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# Cytology, Genetics, and Evolution



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# Cytology, Genetics, and Evolution

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## The Nature of the Gene

By

M. DEMEREC, PH.D.\*

RECENT developments in the studies of the gene make it clear that a gene is an integral part of a chromosome and that it is closely interrelated with the other elements of chromosomal structure. Abundant evidence is available showing that the activity of a gene is determined not only by its chemical constitution but also by its position in the chromosome. Even though I cannot agree with Goldschmidt, who thinks that the interdependence of genes is sufficient evidence for the assumption that genes do not exist, I feel that in considering the question of the nature of the gene it is necessary to treat the gene as a part of the larger structure which is the chromosome.

This discussion will be focused on the problems arising from a detailed cytogenetic analysis of a large number of changes observed in a few regions of the X-chromosome of *Drosophila melanogaster*. These changes were induced by x-raying the sperm in adult males by 2500 to 3000 r-units.

*Types of lethal changes observed at the Notch locus.* For such technical reasons as the ease with which changes are detected, good genetic knowledge of the region and distinct appearance of bands in salivary gland chromosomes, the white-Notch region of the X-chromosome is especially well adapted for cytogenetic studies of changes occurring in genes and chromosomes. Dominant changes at the Notch locus were selected for this study while at the white locus both dominant and recessive changes were studied.

All known Notches are lethal when hemizygous and in the heterozygote they show characteristic notching and delta-like thickening of the veins. Cytological analysis indicates that some Notches are detectable deficiencies while others are not. In our collection of 61 Notch types not connected with chromo-

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somal rearrangements, 46 are detectable deficiencies and 15 are not. Phenotypically, embryologically, and as far as the lethal effect is concerned, these two classes of Notch cannot be distinguished. Both of them show a similar Notch effect, both are lethal and cell-lethal, and as shown by Poulson (1940), both types die at the same stage of embryonic development and show similar embryonic abnormalities.

In deficiencies the Notch effect is undoubtedly caused by the absence of the Notch locus from the chromosome, that is, by the absence of the product which is ordinarily contributed by the wild-type gene at the Notch locus. Since the identical biological effect is evident in Notches which do not exhibit any deficiency, it may be inferred that in both cases the effect is produced by a similar cause, namely by the absence of the Notch gene product. In the case of non-deficient Notches, this absence is probably due to the inactivation of the Notch gene. Thus according to this reasoning the lethal effect of non-deficient Notches is caused by a mutation at the Notch locus which has suppressed the activity of the gene.

This conclusion is based on the assumption that all deficiencies at the Notch locus involve at least one band and are thus cytologically detectable. If generalized this would be equivalent to saying that every locus is represented in salivary gland chromosomes by at least one band and that the non-staining material between two bands does not constitute an essential part of a gene. Decisive experimental evidence justifying this assumption does not exist, and it is not evident how it could be obtained with the methods now available. However, what little is known about the properties of salivary gland chromosomes suggests that bands are in some way related to genes.

One line of evidence favoring the assumption that the bands represent gene loci is the finding that genes are concentrated in euchromatic regions which in salivary gland chromosomes contain many bands, while heterochromatic regions have very few bands and contain very few genes. In comparison with conditions in the mitotic chromosomes, euchromatic regions in salivary gland chromosomes are much longer than heterochromatic regions. For example, in mitotic chromosomes the heterochromatic region of the X-chromosome constitutes approximately one-third of its length, but in salivary gland



chromosomes it shows very few bands and occupies less than one-twentieth of the chromosome length. Similarly the length of the Y-chromosome, which contains a very few known genes, is in mitotic chromosomes approximately equal to the length of the X-chromosome, while in salivaries the length of Y is less than one-twentieth of the X and it contains a small number of bands.

Another piece of evidence favoring the possibility that genes are represented by bands is derived from the study of Notches associated with chromosomal rearrangements. In all such Notches which are available in my collection one of the breaks occurred adjacent to the band considered to represent the Notch locus (3C7), and in all but two cases, no deficiency was detectable. Apparently, non-deficient Notches are more frequent among changes involving rearrangements than among changes not involving rearrangements, probably because the change in position has an effect on the activity of the locus. It is interesting that among 14 rearrangements studied (Fig. 1), 13 had one of the breaks adjacent to the 3C7 band. In 8 of these cases the break was to the right of 3C7 while in 6 cases it was to the left. This evidence indicates a definite field within which the Notch effect may be produced and suggests that the 3C7 band is in the approximate center of the field.

Finally, in the case of Notches as well as in the case of several other loci for which data are available, it was found that whenever a certain band was missing, a certain locus was invariably affected. From what has been mentioned already, it is evident that since a similar phenotypic change may be associated with cytologically detectable deficiencies, as well as with cases where no deficiency is visible, this evidence alone would not be sufficient to connect a certain band with a certain locus. However, when all available evidence is taken into consideration, it seems probable that the bands of salivary gland chromosomes correspond with loci.

*Reaction of different genes to changes in their position.* It is well known that when a piece of a chromosome is transferred through a translocation, inversion, insertion or deletion from its normal position and attached to another region of the chromosomal complex, the genes adjacent to the break are frequently affected. This effect varies. In some cases a viable change is induced and in others a lethal change; also the

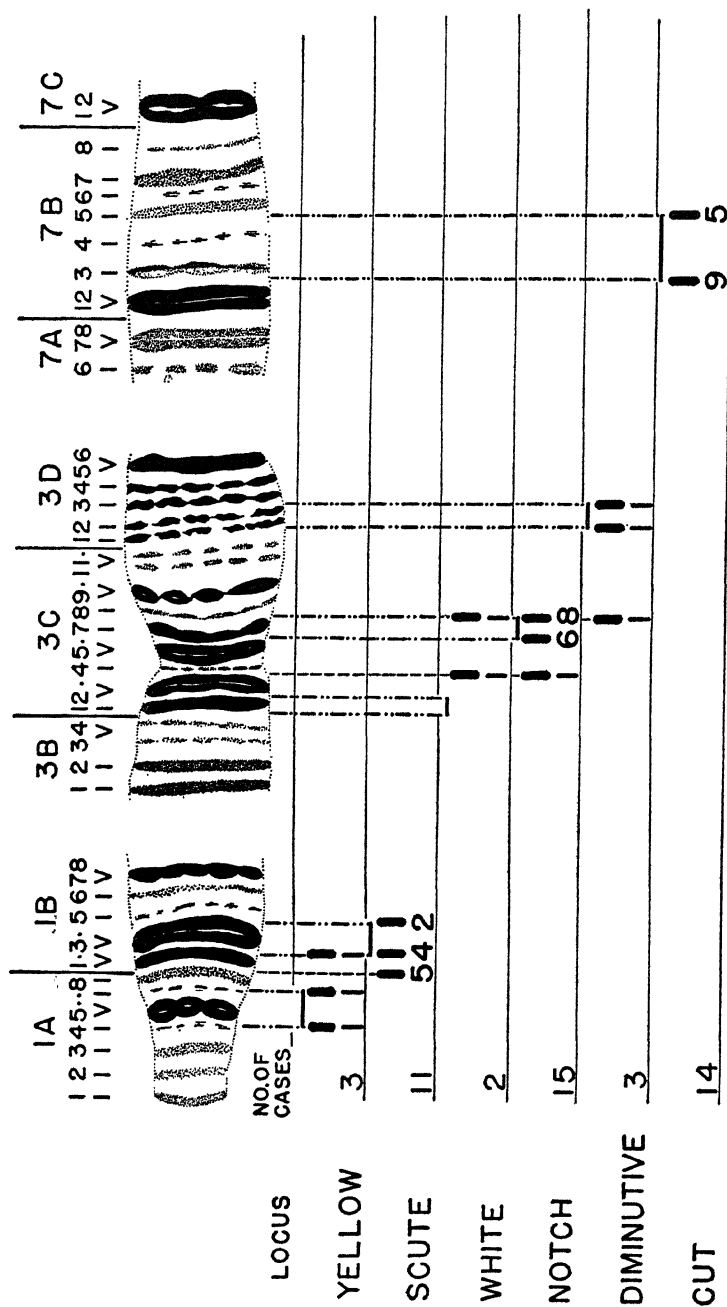


FIG. 1. Location of breaks among mutations associated with chromosomal rearrangements where the recipient section was in the euchromatin. Vertical bars indicate location of breaks and the numbers below show the number of breaks observed; horizontal bars indicate the position of loci.

change may be constant or may be unstable, showing up as mottling. Indications are that the type of change as well as its occurrence is determined by the breakage point near the locus affected and also by the properties of the region to which the piece is attached. The effect of the recipient region is particularly striking in the case of transfers to heterochromatin where the loci involved very frequently show mottling.

In our experiment an appreciable amount of data was accumulated showing the reaction of a number of loci to changes in their position. These data are summarized in figures 1 and 2. The salivary chromosome map (Bridges, 1938) of each region is shown at the top of each figure, and the loci under consideration are listed in the first vertical column; the regions where breaks connected with the change in the locus were found are indicated by short vertical bars and the number under each bar shows the number of breaks observed; horizontal bars indicate the position of the loci.

Figure 1 summarizes the data on rearrangements in which the regions under consideration were reattached to euchromatic sections. It is evident from the chart that an effect was apparent only when the breaks were either adjacent to the locus or in close proximity to it. For example, of the 15 breaks observed in the Notch region, 14 are adjacent to the 3C7 band while only one is separated from it by one double band. It appears from these data that the sensitive region for the Notch locus within which rearrangements involving euchromatin may produce a change in the locus is very short. Moreover the data indicate that breaks adjacent to the 3C7 band are about 14 times as likely to affect the gene as breaks one band distant from it.

The data indicate also that the length of the sensitive region differs in different loci. As measured on the Bridges (1938) map, the length of the sensitive region for the Notch locus is about one micron, for the yellow and scute regions about two microns; and, with only a few breaks available in each, the length of the white and the diminutive regions is more than three microns. Moreover the data for the scute region indicate that a break one band to the left of the scute band is just as likely to affect the scute locus as a break adjacent to it.

However, not all adjacent breaks need produce changes in

loci. We have recorded three breaks adjacent to the scute locus, one break adjacent to the Notch locus, and two adjacent to diminutive, which did not affect these genes. Here again there is an indication that loci differ in their reaction to breaks. Among a relatively small total number of flies examined, 18 lethal changes associated with rearrangements were detected in the cut locus, while among an appreciably larger

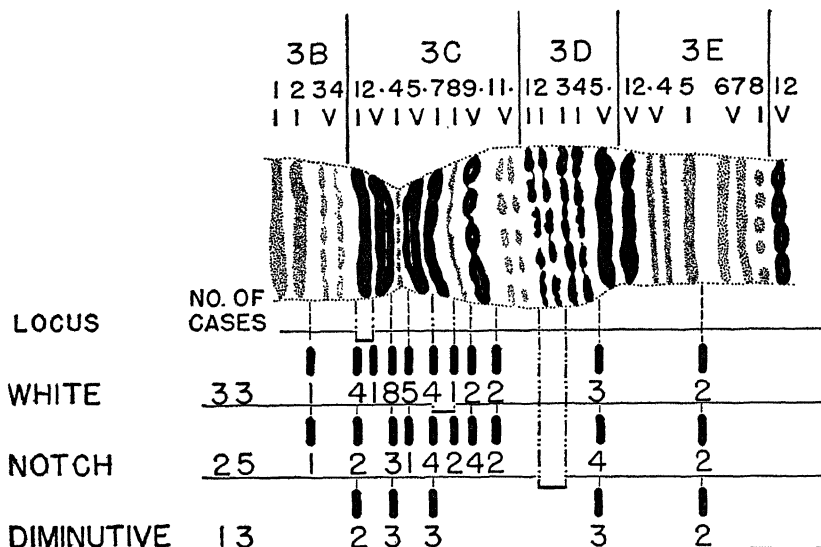


FIG. 2. Location of breaks among mutations associated with chromosomal rearrangements where the recipient section was in the heterochromatin.

number of flies examined for changes in Notch only 14 associated with rearrangements were found. Since the majority of these changes were probably due to shifts in positions, it seems probable that the cut locus is more sensitive to such shifts than the Notch locus.

A striking difference between the behavior of loci when transposed to euchromatin and when transposed to heterochromatin is evident if the data presented in figure 1 and figure 2 are compared. The most obvious difference between the effects of the two regions is in the length of the sensitive regions. Transfers to heterochromatin increased the length of the sensitive region as much as five to ten times. For example, while the sensitive region of Notch in euchromatic transfers is about

one micron, it is about ten microns in heterochromatic transfers.

The number of breaks associated with changes in the white and Notch loci is sufficient to permit a study of their distribution. It should be pointed out, however, that a majority of breaks affecting the white locus affect also the Notch locus; some affect the diminutive locus as well and are listed three times in the chart. Therefore in considering the distribution of breaks each locus should be evaluated separately. It should be pointed out also that the bands 3C<sub>4</sub> and 3C<sub>8</sub> are not distinct and that it is not always possible to determine the exact position of the breaks adjacent to them, so that some of the breaks listed to the left of these bands might have been located to the right and vice versa. The data shown in figure 2 indicate that with reference to the white locus there is an accumulation of breaks between the bands 3C<sub>2.3</sub> and 3C<sub>5.6</sub> while the breaks between 3C<sub>1</sub> and 3C<sub>2.3</sub> are few. Such a situation is unexpected because the low break region is closer to the locus than the high break region. This suggests that the position of the break within the sensitive region and irrespective of its distance from the locus may in some cases determine whether or not the white locus will be affected.

In regard to the Notch locus the distribution of breaks within the 3C region is approximately random, but the accumulation of breaks in one section of 3D and of 3E may be significant to indicate that the Notch locus is sensitive to heterochromatic rearrangements with breaks in these regions.

*Salivary chromosome bands.* Bands in salivary gland chromosomes are aggregates of nucleic acid. They vary in width from 0.2 of a micron to almost one micron. The chromosomes themselves may be considered bundles of chromonemata, as has been suggested by Koltzoff (1934) and Bridges (1935). If it is assumed that in salivary chromosomes the chromonemata are entirely uncoiled then it is evident that each chromonema is composed of light regions not containing nucleic acid and of dark staining parts containing nucleic acid. A single chromonema may be visualized as a bundle of polypeptide fibers such as described by Astbury, held together either through adhesions or through chemical bonds between radicals. Consequently such a bundle of fibers would be composed of regions containing radicals which do not bind with nucleic acid and of radicals

which attract nucleic acid, thus forming non-staining and darkly staining regions. In an aggregate of bundles which make up a salivary gland chromosome, darkly staining regions would assume the appearance of bands.

Bands vary in width from narrow to wide, in intensity from light to dark, in uniformity from evenly stained to irregularly stained, and in shape from full to broken and to capsulated bands. This variability may be accounted for through differences in the nucleic acids responsible for the bands and through differences in the fundamental structure of the protein regions to which nucleic acid is bound.

It has been mentioned already that bands may be from 0.2 to 1 micron wide. In terms of a single chromonema, that would mean that nucleic acid regions are from 2,000 Å to 10,000 Å long. The X-ray study of thymonucleic acid made by Astbury and Bell (1938) indicates that its molecule consists of a long succession of nucleotides spaced at a distance of 3.3 Å which is nearly equal to the distance of 3.5 Å at which is spaced a long succession of amino-acid residues in a fully extended polypeptide. Therefore the side chains of a polypeptide molecule match the side chains of a nucleic acid molecule and in compounds formed between the two the axes of both molecules are parallel. Furthermore, Astbury calculates that the length of the thymonucleic acid molecule in solution is of the order of 6,000 Å, which would contain some 2,000 nucleotides. Consequently the length of the molecule formed by the union between thymonucleic acid and a polypeptide would also be 6,000 Å. An attractive field for speculation is thus opened up, namely that such a molecule may be responsible for a nucleic acid segment of a similar length on a chromonema and a band of a similar width on a salivary gland chromosome. Such a compound between the polypeptide backbone of the chromonema and the thymonucleic acid would account for a medium-sized band 6,000 Å wide. It has been shown by Greenstein and Jenrette (1940) that nucleic acid may have a molecular weight of 500,000 as well as a molecular weight of 1,000,000. This and similar variations in the size of the nucleic acid molecule could readily account for differences in the width of the bands.

As far as the shape is concerned, a band may be: single,

double, full line, broken line, large capsule, two capsules, or a chain of small capsules. These forms are well represented on the salivary maps prepared by Bridges (1938), sections of which are reproduced in figures 1 and 2. A given shape is characteristic and constant for a certain band.

A doublet band consists of two lines similar in appearance which are usually parallel and frequently are located so close together that only in very good preparations can they be distinguished as separate lines. In our material a break between two lines indicated by Bridges as a doublet has never been observed. In sections 3B and 3C of the X-chromosome, breaks were found between all bands but never within a doublet. As far as its origin is concerned, a doublet may be visualized as formed by two either identical or very similar molecules containing nucleic acid and separated only by a very small section of the polypeptide chain not bound with the nucleic acid.

A large capsule is composed of a pair of doublets the ends of which either come into contact or are fused. Since the bands on the map are drawn at one optical level, most likely at the middle of the chromosome, the picture so prepared represents a cross section through a band. Actually each line on the map is a disk extending through the chromosome and in the case of a capsule, the edges of two disks are fused all around. Since it is known that there is a strong attraction between homologous bands, such joining on the periphery of the elements of two adjacent bands may be expected to occur whenever these bands are sufficiently similar to cause attraction.

In a full line the nucleic acid appears continuous throughout the whole optical section, which means that in such a line the nucleic acid regions of individual chromonemata adhere together and form a disk. These disk-like structures are recognizable in stained preparations.

The origin of a broken line may be explained by the assumption that the adherence of nucleic acid regions does not involve the whole complement of chromonemata but is limited to bundles involving only a certain number of them. In salivary glands, as a rule, homologous chromosomes are synapsed and therefore each chromosome consists of two homologous chromatids. In suitable figures it is possible to recognize tetrapartite structure indicating that at the time of synapsis each chromatid was already split into two sister chromatids. Now if

the fusion between nucleic acid regions is limited only to the bundle of chromonemata derived from one chromatid such a condition would produce a line consisting of four segments. Lines with two segments would originate if only the chromonemata derived from two sister chromatids fuse into one disk so that two disks corresponding to two parental chromatids are formed. Lines with more than four segments could be formed through breaks in the fused bundles after they reach a certain size.

It has been mentioned already that identical bands have a tendency to synapse and if the two bands of a doublet are identical, the peripheries of two disks forming a doublet would synapse and a capsule would be formed. Similarly, if the bands of a doublet are segmented, synapsis would be expected on the periphery of every segment and a double capsule in case of a two-segmented band or a chain of capsules in case of multisegmented bands would be formed.

*Conclusion.* In conclusion the main points presented in this discussion may be summarized as they are related to the study of the gene.

An intensive cytogenetic study of a large number of changes affecting several loci in the X-chromosome of *Drosophila melanogaster* indicates that there is a sensitive region on each side of a gene. If the chromosome is broken within this sensitive region and the broken segment attached to some unrelated section, the gene is frequently affected. The effect is probably caused by the change in position and may be due either to a change in the gene or to a change in gene activity. The length of the sensitive region varies with the locus and is also influenced by the nature of the recipient section. As a rule, the sensitive region is longer when heterochromatin is the recipient.

A salivary gland chromosome may be visualized as a cable of fully extended chromonemata, and a band as an aggregate of nucleic acid. Experimental evidence suggests that a gene is located within a band. If it is assumed that a chromonema is composed of fibers having a polypeptide backbone, the bands would represent the regions where molecules of nucleic acid are attached. Recent studies made by Astbury (1938) indicate that the length of a thymonucleic acid molecule is about 0.6 of a micron which is of the same order of magnitude as the



width of a salivary chromosome band, and also that the spacing between nucleotides matches the spacing between side chains of polypeptides and that presumably nucleic acid is able to form bonds with the polypeptide fibers. Thus an attractive possibility is open for speculation that each salivary band may consist of a single molecule which is repeated a great many times, the length of the molecule determining the width of the band.

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## Chromosome Structure

*By*

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I FEEL very much complimented to be invited to discuss the subject of chromosome structure here, for although I have done some work in this field, I do not pretend to be a special authority on the subject. This latter fact, however, may have a certain value in that it permits me to approach the problems with at least a reasonable degree of objectivity—which seems particularly desirable in the present state of the subject. What preconceptions I may have from the study of the giant salivary gland chromosomes of *Diptera* should not unduly influence the discussion because I feel that the salivary gland chromosomes are highly specialized bodies and that it is uncertain how far evidence from them can be applied to the organization of “ordinary” chromosomes. This last statement will also indicate why it seems desirable here to center attention primarily on “ordinary” or “orthodox” chromosomes (capable of mitosis and normal functioning) rather than on the giant chromosomes.

Since it is impossible to treat all aspects of the subject in a discussion like this, let me make clear at the outset just what is to be attempted. Our primary concern is with chromosomes and heredity. Consequently, topics must be omitted which are primarily of cytological interest, such as those dealing with chromosome mechanics and with accessory structures like nucleoli, chromosome sheath, chromosome matrix and the so-called spindle fiber attachment body. We are interested in the essentially permanent gene-bearing part of the chromosome—that is, the chromonema—and attention will be confined primarily to that. I should first like to outline some of the main facts thus far brought out concerning the nature of chromonemata, and then to turn briefly to two of the problems which have not yet been solved, but which seem to be especially inter-

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esting in the present connection. Naturally, there is no chance to go into details in this account. The subject can be presented only in broad outline.

Ordinarily, when we speak of a chromosome group we mean something like that shown in figure 1. This happens to be the chromosome group of a male robber fly (*Dasyllis*). The chromosomes of many other animals or plants would serve just as well. We know, of course, that the chromosomes here are in pairs, and that one member of each pair comes from the mother and the other from the father. In this particular case they exhibit paired association. Likewise, we know that the two members of each pair are homologous and have essentially the same sets of genes. The only exception is the unpaired X or sex chromosome, which is responsible for sex determination, as was discovered long ago by Professor McClung.

Our concern is with what is inside these chromosomes, any of them. Under proper conditions, in favorable material, it is readily seen that the frankfurter-like metaphase chromosome is really made up of one or more threads coiled up like a screen door spring (Fig. 2). These are the chromonemata.

Although chromonemata were described as early as 1880 by Baranecki, were re-described in the period 1908-1912 by Bonnevie and by Vejdovsky, and subsequently have received attention from a whole series of investigators, we are still far from having any clear conception of their structure. The main reason for this lack of knowledge, of course, is the fact that they are so small. They are only visible under the microscope. Consequently, they cannot be taken apart and analyzed directly. In diameter they often approach the limits of visibility even under the highest powers of the microscope, with the result that a study of their internal structure by direct observation is exceedingly difficult. This fact helps us to understand why our knowledge of chromonemata is limited and also why many divergent conceptions have arisen regarding their structure. Our task is to put together the various lines of evidence and to try to get some general conception of what a chromonema is. Fortunately, others have had the same task, and I have been able to profit greatly from recent reviews of the subject, especially those of Nebel (1939) and of Kuwada (1939).

Before trying to form a conception as to the internal structure of chromonemata it is necessary to consider their external char-

acteristics, and this leads us directly into two recent developments which should, perhaps, be considered at the outset. When chromonemata were first discovered, and for a long time afterward, it was thought that what appeared under the microscope to be a single chromonema in a chromosome was indeed such. More recently, however, Nebel and several others have produced impressive evidence to indicate that in many cases such a supposedly single chromonema is in reality two or even four closely applied chromonemata (see Nebel, 1939, for references). Particularly convincing evidence bearing on this point has recently been secured by Hughes-Schrader (1940) in her work on the coccid, *Llaveiella*. In this material, during spermatogenesis, the chromonemata dissociate into clear cut chromosomes and show that each original chromosome contained "at least four chromonemata at metaphase." It is also stated that in some cases there is clear evidence of an additional split, which would make eight chromonemata in the original chromosome.

This is rather disconcerting, from our present standpoint, because most of our ideas regarding the internal structure of chromonemata have been based on the assumption that we were looking at single elements. Perhaps even more disconcerting is the fact that several recent workers, especially Fujii, Kuwada and their associates in Japan (see Kuwada, 1939) have produced considerable evidence to indicate that the coiled chromonema, as we usually see it, is not a simple structure, but is itself made up of a secondary, very delicate and close coil. In other words, the relatively heavy thread which we see may in reality be made up of an extremely delicate and much longer thread, itself tightly coiled in a very fine spiral, as indicated schematically in figure 3.

These two complications together, it seems to me, put in question much of the evidence which we have long been using to indicate how a chromonema is constructed, for obviously if we are trying to analyse the internal structure of what we see under the microscope as a single thread, it makes a deal of difference whether the thread is really single or is double or quadruple, and whether it or each of its component threads is simple or is itself coiled into a fine, almost sub-microscopic spiral. Attention is called to these two complications at this point, so they can be kept in mind as we turn now to some of the well-known evidence concerning chromonema structure. It

should be evident as we go along that the significance of the structures seen in a so-called chromonema can hardly be determined with assurance until these two features have been cleared up.

Some of the most favorable material for study of chromonemata is found in maturing germ cells, at the leptotene or pachytene stages. Here the chromonemata are relatively large and are extended into relatively straight threads; at least the large coils are straightened out. In ordinary fixed material the threads exhibit granules or chromomeres scattered along their length, and as Professor Wenrich pointed out in his now classic paper of 1916, each chromosome may have its own relatively permanent pattern of chromomeres—indicating that the chromomeres reflect in some way a qualitative linear differentiation of materials in the chromonema. Belling (1931) has even gone so far as to suggest seriously that the more delicate or “ultimate” chromomeres, visible in such favorable material as the lilies (Fig. 4), actually represent individual gene loci. Especially illuminating from our standpoint is the coöperative work on gene localization in maize (as yet largely unpublished) in which cytological and genetic methods have been combined. This has shown that in maize individual chromosomes not only have recognizable chromosome patterns, but that the approximate loci of several genes correspond to the positions of individually recognizable chromomeres. The large chromomeres involved here have a constancy which can hardly be questioned. These, however, as we will see later, are presumably compound structures. As regards the significance of the small granules there is considerable uncertainty, not only because of the difficulties already mentioned, but because of irregularities in size, number and position which have led some authors (e.g., Randolph, 1932) to consider them as probably artifacts.

The most striking and clear-cut evidence of longitudinal differentiation in the chromonema, and its relation to gene loci, is seen in the giant salivary gland chromosomes of *Diptera* (Fig. 5), particularly in *Drosophila* where, through the discovery of Painter, and subsequent work of Bridges and many others, accurate mapping of the gene loci in terms of visible differentiation within the chromosomes has been carried out (see Bridges, 1935).

This evidence from salivary gland chromosomes, combined

with the earlier evidence reviewed above, makes it seem clear that the linear chemical differentiation within the chromonema, representing the gene sequence, is reflected in morphological characteristics which become visible under the microscope in fixed and stained material. In a general way, of course, this is simply a restatement of the conception which has been recognized for many years, and is a verification of the hypothesis presented by Roux as early as 1885 on the basis of the accurate longitudinal division of the chromosomes. I have reviewed it because it is a necessary foundation for any advance, and brings us face to face with some of the problems which have not been solved.

Up to this point we have been on reasonably solid ground; but from here on we are apt often to leave the ground altogether. I have already suggested some of the difficulties and differences of opinion. Probably you will not all agree with the rest of what I have to say, even if you have agreed thus far. But that is as it should be, it seems to me, for as long as we are not prepared to give final answers, all views should be welcomed.

The remainder of the paper will be devoted to a very brief consideration of two problems. These problems are closely related; but for practical purposes are distinct. One concerns the longitudinal organization of the chromonema—the other concerns the transverse organization. The former has already been considered somewhat. As indicated, however, the evidence of differentiation which we have reviewed thus far does not serve to tell us with certainty, or in specific terms, how a chromonema is constructed, what the chromomeres really are, how much, or what parts, of the chromonema should be regarded as gene-bearing material, or how the genes are held together. These are some of our ultimate problems, and we are far from having them solved.

It is simplest to take up the question of the chromomeres first. We have already seen that, e.g., in maize, the conspicuous chromomeres make a fairly constant pattern. But it seems clear from genetic evidence that there are not anywhere near as many of these chromomeres as there are genes—so they must be compound. Evidence from the salivary gland chromosomes indicates the same thing. There are many more cross bands in the latter than there are chromomeres in an ordinary chromosome. It seems highly probable, therefore, that most chromo-

meres include the equivalent of several of the differentiated bands or disks in the salivary gland chromosomes, and hence that the ordinary chromomere pattern can hardly be more than a crude representation of the real linear differentiation.

But what about the bands in the salivary gland chromosomes? Do they represent the ultimate genic "units"? In attempting to answer this question it might be recalled first that the pattern here consists of alternating chromatic and achromatic regions, and that there is not yet agreement as to whether the genes are in chromatic or achromatic materials. Koltzoff (1938), for example, holds to the latter alternative. Since most attention has been given to the chromatic "bands," however, we may inquire as to the morphological significance of these. So far as the heavy bands are concerned it seems clear that they are not longitudinal units, for they appear to be compound and made up of thinner bands, which under proper conditions are visible as such. Then what about the thin bands? Some of these go down practically to the limits of microscopic vision and give the appearance of being single. Here again there is a difficulty, however, for we have no final criterion for "singleness" in such a case and, furthermore, it is a serious question as to whether these delicate disks are really constant. In my experience some of them, at least, appear not to be constant, and I think most other students of these chromosomes have had a similar experience.

In summarizing the lines of evidence just reviewed, therefore, it may be stated that, although the goal may not be far off, we still have not reached the stage at which we can point to microscopically visible structures in the chromonema and say with assurance, "These are the ultimate genetic units."

But suppose we leave out the question of size and consider only how the units are held together. Genetic evidence, from crossing over, etc., seems to indicate that a definite boundary or gap exists between each two successive genes in the series, and that when a transfer occurs, the "break" regularly comes at this gap. In extreme form this would lead to the well-known string-of-beads type of picture. Of recent years, however, there has been a tendency to get away more and more from such a conception and to lean toward the view that an intimate connection, and perhaps interdependence, exists between adjacent genes. This shift in view has come, in part, from the discovery of the "position effect" which indicates that, in some cases at



least, the behavior of a gene depends on its neighbors and changes with a shift in position.

Before leaving this topic, however, we shall turn to another line of cytological evidence which points in the opposite direction. This is provided by the so-called "lamp-brush" chromosomes found in the oöcytes of numerous vertebrates (Fig. 6). These classic structures have just begun to receive the attention they deserve. Fortunately they are fully functional, germ-line chromosomes, and it is legitimate to apply evidence from them directly to the problem of chromosome structure. Since these chromosomes are discussed (from a somewhat different angle) elsewhere in this series by Dr. Duryee, only a few points will be referred to here. According to the accounts of Koltzoff (1938) and of Duryee (1941), which are in essential agreement, the very large, extended chromonemata here actually appear like strings of beads, even in the living or very near the living condition. Furthermore, each chromomere apparently undergoes a series of longitudinal divisions in the germinal vesicle, producing a lateral branch, itself made up of chromomeres like the original. These branches give the lamp-brush effect; later they all fall off. It seems especially significant that these lateral branches are not connected longitudinally. Linear continuity is maintained only in the original chromonema. If the evidence here is correct, it seems to indicate strongly that the chromomeres of a chromonema possess a high degree of independence. The problem demands thorough reinvestigation to establish exactly the history of the side branches and to ascertain whether or not the chromomere pattern for individual chromosomes is constant. Painter (1939) has suggested, from analogy with multiple chromosome complexes in the *Diptera*, that the side branches here are whole chromosomes, not branches; but if the evidence of Koltzoff and of Duryee is correct, this interpretation cannot hold.

This leaves us in a very unsatisfactory state so far as the longitudinal organization of the chromonema is concerned. Now what about the transverse organization? From the genetic standpoint this is an important topic. Originally, I believe, the chromonema was supposed to have a uniform or simple type of structure transversely at any one level and was supposed to reproduce by longitudinal splitting. More recently, however, there has been a strong tendency, especially among geneticists,

to picture the chromonema as a submicroscopic gene filament surrounded by a relatively thick covering of non-genic chromatic material, and to assume that the gene filament reproduces by laying down a new one alongside itself (cf. Snell, 1938). This conception applies irrespective of whether we think of the chromonema as a uniform thread or as a string of beads.

Without attempting to speak finally, I must confess to a preference for the older type of view. This is not intended to imply that the chromonema consists entirely of genic material, even at any one locus, but rather that the genic material is interspersed with the non-genic material and is found at the periphery just as well as in the interior of the chromonema. In a molecular sense the structure as a whole would, on this view, be multiple in cross section. The reasons for such a view are based on behavior, which in this case I think is more reliable than structural evidence. The primary phenomenon of interest is synapsis—the intimate side-by-side union of chromonemata. In spite of the fact that under certain conditions non-homologous association may be detected (McClintock, 1933), it seems clear, as has often been pointed out, that the underlying basis of synapsis is the likeness of homologous genes. Ordinarily, synaptic union is attributed to an attraction of like for like. For example, Muller (1937) has postulated that each gene sets up a specific field of attractive force, resulting in a “specific auto-attraction” between like genes.

Assuming that such an attraction exists, it is very difficult for me to conceive how a chemical unit, the gene, of sub-microscopic proportions, could set up a sufficiently powerful field of force to act through the supposed thick chromatic hull (not to mention the distance between separated chromonemata), and thereby bring together the homologous parts. This difficulty is not lessened by the possibility that at the time of synapsis a special nuclear mechanism may operate to bring homologues into contact at one or more points and that from here synapsis may progress zipper-like along the threads. Molecular attraction would presumably act through a distance of a few Ångströms, while the thickness of the “hull” is apparently to be expressed in fractions of a micron, at least in many organisms. The suggestion has been made (e.g., Snell, l.c.) that the postulated hull material surrounding each gene is different from that surround-

ing the others, and that this material may exert the "attraction." I fail to see any real advantage in such an assumption. The only way we can legitimately assume that hull materials could all differ from one another in the required manner is to assume that each gene produces a specific kind of chemical substance around it, different from all others. The simplest way to account for such a condition would be to assume that the basic genic material at any one locus simply multiplies or reproduces itself. In that case the genic material would lie at and near the periphery as well as in the axis of the chromonema or chromomere. There would not be an outside hull of one special chemical constitution and an infinitesimal inside gene of another special chemical constitution. To assume the latter seems merely to complicate the situation.

As a third possibility, it has been suggested by Lindegren and Bridges (1938) that there is no synaptic attraction, but that the union is brought about by a serological reaction—an agglutination produced by antigen-antibody union. The considerations just outlined apply equally well to this suggestion. If synapsis is an agglutination reaction, it should mean that at their surfaces the gene loci (chromomeres?) all differ from one another. To assume that this reflects a difference in non-genic materials, manufactured by the individual genes, is more complicated than to assume that the genic materials themselves extend to the surface and are directly involved.

Regardless of which view we take of synapsis, therefore, we are led to regard a chromonema as composed, in cross section, of a mixture of genic and non-genic materials. The genic materials could be in the form of chain molecules, cyclols or a fabric (see Wrinch, 1936), but there should be many molecules of a kind at any locus and they should be found as much near the periphery as in the axis, presumably interspersed with nucleic acid and other constituents.

It should be observed, of course, that the type of view just favored presents some difficulties when one tries to account for the process of mutation; but in this respect it is perhaps no worse than others. With our present lack of knowledge as to what mutation is, there is no evidence, so far as I know, which rules out such an interpretation.

Another question which may readily be raised is: How, on

the view just outlined, do the genic materials and the chromonema reproduce? It may be answered that the individual molecules of genic material could reproduce by laying down new ones alongside themselves, but that the chromonema as a whole or the chromomeres (?) would reproduce by longitudinal division, not by laying down a new chromonema or new chromomeres.

Perhaps, in this connection, it may be profitable to say a word about the question of chromosome sizes and its possible bearing on the problem just considered. It is a remarkable fact that in some groups of organisms the chromosomes are enormous, as compared with those in other groups. Yet, so far as I am aware, there is no direct evidence of a corresponding difference in the number of genes or in the genetic complexity in such cases. Figures 7 and 8 illustrate the point. The former is from a preparation of *Trillium grandiflorum* loaned by Dr. B. R. Nebel, the latter from a preparation of the fungus, *Psalliota campestris*, loaned by Dr. H. G. Hutchinson. They are made at the same magnification. The entire nucleus of the fungus is minute, compared with the chromosome group of *Trillium*. In the fungus it is doubtful if a single, extended chromonema would be visible even under the highest magnification. Does this mean that in such a chromonema there is a sub-microscopic filament of genes, with almost nothing around it, while in *Trillium* there is a similar filament with a thick hull around it? If so, why is a hull necessary in some organisms and not in others?

Combining all these considerations and others which I have not had time to mention, it seems to me most satisfactory, as a working hypothesis, to regard chromonemata as fundamentally alike in structure in different organisms and as constructed in the manner outlined above. There may be thousands of similar molecules or linked groups of molecules at one locus, but the number need not be the same in different cells or in different organisms. Thick chromonemata should contain more genic material than thin ones of the same length, and should, in general, be found in larger nuclei. And the degree of thickness which would ordinarily induce longitudinal splitting of the chromonema could differ widely, both within an individual organism and in different species.

It may be noted in passing that the considerations reviewed here regarding chromonema size and possible synaptic attraction appear to apply equally well to the phenomena of induced chromosome rearrangement. In interpreting induced rearrangement, many authors consider that the chromonemata are actually broken in two by individual ionizations and that the free ends then possess an attraction for one another which causes them to reunite in the same or in new combinations. Rearrangement is induced readily in organisms having what appear to be large, thick chromonemata (e.g., *Tradescantia*, *Orthoptera*) as well as in those having thin ones. It has always seemed improbable to me that structures of such dimensions could thus directly be broken. Likewise, it is difficult to see how broken and separated ends could possess non-specific, mutual attraction operative through the distances which seem to be required in such cases. It would seem more probable that if a chromonema is once completely broken and the ends separated, it remains broken. Individual ionizations might cause localized alterations (e.g., solation of the matrix) in the chromonema or its surroundings, some of which ultimately lead to breaks, and some of which ultimately lead to rearrangements (perhaps by a process comparable to crossing-over; see e.g., Belling, 1931); but it does not seem necessary as yet to assume that breaks lead to rearrangements. We are very much in need of critical evidence on this point. Such evidence is hard to get, however, because most results can be explained on either view.

## CONCLUSION

As you have seen, this discussion has not given a clear idea of what a chromonema is. No one knows just what a chromonema is or how it is constructed; so if the picture we have obtained in this discussion is vague, it is perhaps still in keeping with the present state of the subject. Our conception of chromosome structure, like our conception of genes and of mutation, has been going through a shaking-up process recently and, until the process is complete, any interpretations, including those treated here, should be held with reserve. My main plea is that we do not be too hasty in throwing out hypotheses which, like the one considered in the latter part of the present discussion, may yet prove to be very useful.

FIG. 1. Photomicrograph of spermatogonial chromosome group of the robber fly, *Dasyllis* sp.?  $\times 2500$ .

FIG. 2. Photomicrograph of a prometaphase chromosome (tetrad) at the first meiotic division in *Trillium erectum* L., showing coiled chromonemata. (Composite of two focal levels.) From a photograph kindly loaned by Prof. C. L. Huskins. See Huskins and Smith, 1935, Fig. 25c.  $\times ca\ 2400$ .

FIG. 3. Diagram representing the delicate spiral considered by some authors to be present within the main spiral of the chromonema. See text for explanation. From Kuwada, 1939, Fig. 1d.

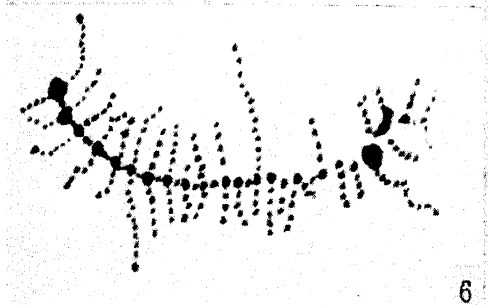
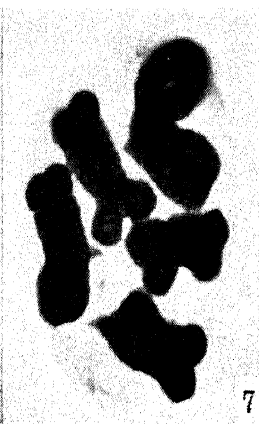
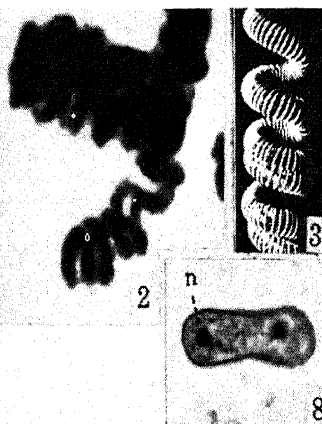
FIG. 4. Pachytene chromosomes of *Fritillaria purdyi*, showing chromomeres. Photomicrograph, by the author, from a preparation kindly provided by Dr. John Belling.  $\times 1400$ .

FIG. 5. Photomicrograph of a portion of a salivary gland chromosome of the fungus fly *Sciara ocellaris*.  $\times 1700$ .

FIG. 6. Portion of a "lamp-brush" chromosome from ovocyte of the fowl. From Koltzoff, 1938.

FIG. 7. Chromosomes of *Trillium grandiflorum*. Metaphase of first meiotic division in pollen mother cell. Photomicrograph by the author from preparation kindly loaned by Dr. B. R. Nebel.  $\times 850$ .

FIG. 8. Nuclei of the common mushroom, *Psalliota campestris*. Photomicrograph by Dr. Warren H. Lewis, from preparation kindly loaned by Dr. W. G. Hutchinson.  $\times 850$ .







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The Sex Chromosomes: Heteropycnosis and  
Its Bearing on Some General Questions of  
Chromosome Behavior

By

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THE discovery by McClung in 1901 that certain chromosomes—sex chromosomes—stand in a causal relationship to the process of sex determination is one of the outstanding accomplishments of the present century. The cytological mechanism that is involved has since been worked out in most of its important aspects. The geneticists have determined the part played in the process by other chromosomes as well as sex chromosomes, and the physiologists are now rapidly elucidating by what means the basic mechanism expresses itself phenotypically. At least for the present, the work of the cytologist on this old problem has been completed.

However, it has now become clear that the sex chromosomes point the path toward a solution of many other questions which bear no direct relation to sex determination at all. The cytologist has come to realize that the sex chromosome is fundamentally not different from the other chromosomes, the autosomes, and that its special features are due to the accumulation and perhaps exaggeration of certain structural elements, differences in timing of some of its cyclical changes, and adaptations to the lack of an homologous partner in the heterogametic sex.

One of the special features which may prove to be of the utmost importance in elucidating the physiology of chromosomes in general is *heteropycnosis*. In its outward aspects it is most strikingly encountered in certain insects such as the Orthoptera and Hemiptera, where in the males the sex chromosome has no homologous partner, and where, during the meiotic prophases it has the tendency to appear more dense and to

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stain more intensely than the autosomes. This striking peculiarity has aroused curiosity ever since its discovery, and consequently much work has been done on it. We have since found that other chromosomes frequently also have this property, though in nearly every case it is not developed to such an extreme degree and is usually confined to relatively small sections of the chromosome body. The explanations that have been offered for this behavior cover a wide range and none of them is entirely satisfactory.

Thus Mohr (1915) believes that heteropycnosis results because in the heterogametic sex the sex chromosome undergoes the mitotic process without an homologous partner. Despite much criticism to which this suggestion has been subjected, it may well be valid and correct so far as it goes. It will, however, be realized that it represents only a small initial step in an analysis of the problem, for it will at once be asked just why the presence of an homologous partner should affect the process of condensation.

Heitz (1935) has given us very valuable information on heteropycnosis, especially in autosomes, and concludes that it is conditioned by a special type of chromatin—heterochromatin. In other words it is due to morphologically different material present in certain regions of some chromosomes. The evidence seems clear, moreover, that such regions are more or less inactive, genetically speaking. But as Heitz himself points out, other features are also involved. Thus in most Hemiptera heteropycnosis is not observed in most cells of the homogametic sex, and indeed even in the heterogametic sex it is often encountered only during the meiotic period. Clearly, therefore, the behavior of heterochromatin is considerably affected by conditions that are in a sense extraneous to the chromosome.

Wenrich (1916) has emphasized that heteropycnosis involves not so much a special kind of process but rather the degree and chronology of certain changes which autosomes also undergo. This is peculiarly applicable to the Orthoptera which Wenrich studied, but it is an explanation not easily utilized in cases like Heitz's plants, unless one postulates that the degree of change implies also a morphological basis (in the cytological sense).

Without representing a complete survey of the question the foregoing will serve to show that its present status is one of

preparation. For the hypotheses mentioned are not mutually exclusive, and it is more than likely that they will all play a part in the final solution. Almost any new finding should help to clarify the issues involved, and it is with this in mind that some special aspects of heteropycnosis are here taken up.

One such aspect is comprised in the special behavior of heteropycnotic chromosomes with respect to each other. The sex chromosomes of a bug like *Brochymena* (Wilson, 1905) and many other Heteroptera may serve as an example. The male has a large X and a small Y. Both are heteropycnotic during the meiotic prophases and are easily recognized from the resting stage on. During the synizesis or synapsis stage, these two chromosomes come together in close contact, or, more often, form a single densely staining body. Before late diakinesis they separate again and divide equationally in the first division, showing no relation to each other during the process. After the late anaphase and before the second metaphase, however, they once more approach and come into contact with each other—but this time only momentarily. In the division that follows they go to opposite poles—that is, they divide reductionally. Similar behavior has been noted in many other insects.

There are thus two distinct periods during meiosis when an attraction between the X and the Y is evinced—approximately at synapsis, and again just before the second division. Occurring at about the time when the autosomes also are subject to forces of pairing, the first of these might well be considered to be of the same nature. But if the interpretation given by Darlington (1937) for such pairing (synaptic attraction is satisfied by the union of chromosome elements in twos) be followed, it will be seen that the sex chromosomes present various difficulties. For instance, it is beyond doubt at least in certain cases that both X and Y are already split before they come together, and they would thus not conform to the basic rule of such pairing. If, however, the union is attributed to forces of "secondary pairing," nothing more is gained than a name for forces about which we know nothing.

The temporary union of X and Y prior to the second division presents similar difficulties, if appeal be made to the forces involved in autosomal pairing. Darlington suggests that this union is perhaps due to a property analogous to terminal affinity, but

I am not sure that that is not tantamount to saying that we don't know how to explain it.<sup>1</sup> It is my contention that we are indeed confronted here by forces not directly involved in autosomal pairing, and that these forces are correlated with the factors that bring about heteropycnosis.

The argument that can be made for this hypothesis on the basis of the sex chromosomes of Heteroptera such as I have just described can be extended in several ways. There is, for instance, the behavior of compound sex chromosomes as encountered in certain Reduviidae. In the uncomplicated case of an X chromosome of two components and a single Y, these all come together in the synizesis to form a single, intensely staining body. Before the first metaphase they separate again to divide quite independently of each other, only to attain approximation once more just before the second metaphase. In short, their behavior duplicates that of an orthodox XY condition, nor is the process altered when the number of X components is raised to 3, 4, or 5. If the compound X represents merely a single chromosome broken into a number of smaller ones, it does not seem likely that we are dealing here with the forces that cause pairing of genetically homologous chromosomes. The only feature that all of these chromosomes have in common is heteropycnosis.

The same principle seems to hold in certain scale insects like *Pseudococcus* (Schrader, 1923). The conditions here are not yet fully analyzed, and it is sufficient to say that in the males half of the chromosomes are heteropycnotic and come into close contact with each other during the first meiotic prophase. This association becomes much less intimate or is broken altogether before the first metaphase, only to be regained before the second anaphase. Almost certainly we are not dealing with compound sex chromosomes here, but the chromosomes involved are heteropycnotic, and as such they follow the same

<sup>1</sup> However, in a paper recently published Darlington (1939) explains this puzzling behavior by what may be termed largely extra-chromosomal forces involved in the mechanism of mitosis. His argument rests on such suppositions as a "change in precocity" of pairing, metaphase configurations as determined by minimum distances between chromosomes and spindle size, valency and conditions of polarization of the centromeres of the sex chromosomes, and the size of the chromosomes. The hypothesis as such is admirably thought out, but until Darlington offers more factual evidence in support of most of the assumptions involved it had perhaps best be held in reserve.

steps that heteropycnotic sex chromosomes of an entirely different category go through.

The behavior of supernumeraries in *Metapodius* should also be considered. Wilson (1909) justifiably concluded that these chromosomes represent extra Y's, and some individuals may thus have as many as seven instead of the normal single Y chromosome. These supernumeraries are heteropycnotic like the regular XY pair. Furthermore, all supernumeraries and the normal sex chromosomes tend to come together at the time of synizesis. As in the other instances listed here, they behave independently in the initial stages of the first division, and preparatory to the second division are once more subject to collocation. Here then is a case in which the participating chromosomes are, or were originally, homologous; but by the same token the factors that underlie the meiotic union of chromosome elements in pairs cannot be involved, for there may be 3, 4, 5, 6, or 7 such chromosomes, all attracted to each other.

That brings up the question of the salivary or polytene chromosomes. It is clear that in various species of *Drosophila* every chromosome carries a certain amount of heterochromatin, usually located near the spindle attachment. The salivary chromosomes represent prophase chromosomes, and all their heterochromatin sections follow the rule observed in the preceding cases—they tend to come together during prophase. Hence arise the characteristic masses of heteropycnotic chromatin from which radiate those sections or arms of the chromosomes that are euchromatic and that are not subject to the influence that brings about a union of the heteropycnotic material. Bauer (1936) states that such "interchromosomal union is determined by a specific attraction of certain heterochromatic sections," and thus arrives at a conclusion for the salivary chromosomes which may well have a much wider application.

### THE CASE OF *RHYTIDOLOMIA SENILIS*

The contrast in behavior between heterochromatin and euchromatin is brought out in a rather unique way in a bug whose primary interest for me lay in another direction (Schrader, 1940).

Both sexes have a diploid number of six chromosomes, the

male being heterogametic and carrying an XY pair of sex chromosomes. The Y is somewhat, but not much, smaller than the X. After the last spermatogonial division, the preleptotene stages show the heterochromatin of the two sex chromosomes as two rounded chromatin nucleoli, and later there is also a large, less intensely staining plasmosome.

Rhytidolomia shares with other Heteroptera those conditions which make a study of synapsis very difficult. However, it is approximately at this time that the heteropycnotic chromatin represented by the two chromatin nucleoli frequently becomes joined or fused into a single body (Figs. 1 and 2). Apparently such union occurs only during this period of the prophase, although the resolution of the resulting body into its two constituents may occur at any point from the beginning of diplotene to middle diakinesis.

In any case it is clear already in early diplotene that this heterochromatin is always connected with euchromatic threads (Figs. 3 and 4), a first indication that the sex chromosomes are composed of both types of chromatin. Early diakinesis furnishes evidence that each sex chromosome has two such euchromatic threads (Figs. 3, 4, and 5) which differ in no discernible feature from the autosomal threads.

As has been reported elsewhere, the meiotic pairing that may have occurred during the synapsis stage is abrogated by the time the diakinesis is reached, and the diploid number of chromosomes, each composed of two threads, is then easily demonstrated. By the time of middle diakinesis the heteropycnotic sections of the X and Y have always separated from each other, and their euchromatic ends evince an activity that corresponds exactly to that of the autosomes. This activity takes the form of an end to end approach culminating finally in a terminal union (Figs. 5 to 8) between homologous chromosomes. In the sex chromosomes as well as in the autosomes a fine fiber frequently appears between these ends as they come toward each other. There is no doubt that the euchromatic ends of the sex chromosomes behave in every way like the autosomes, and the three tetrads of the first metaphase thus all arise in the same way.

The final condensation leaves both X and Y club-shaped, with the terminal union occurring at the narrow euchromatic



ends while the heterochromatic region is represented in the distended distal ends (Figs. 9 and 10). The autosomes have no such differentiation in shape. On the first metaphase plate polar views show that the X and Y always form an obtuse angle with each other (Fig. 9).

The division of the sex chromosomes that now follows has been described by Wilson (1913) and confirmed by me. As in most Heteroptera it is equational for the X and Y, the separation beginning at the heteropycnotic ends (Figs. 10 to 13) which lead the way to the poles.

There is practically no interphase, but it is then or perhaps even earlier at the end of telophase, that the sex chromosomes execute a significant manoeuvre. During the first division they usually become slightly separated but they now approach each other again. This approach and their final contact now, however, occur at the blunt, heterochromatic ends (Fig. 14). It is a typical touch-and-go movement, as seen in the sex chromosomes of many other Heteroptera. As the X and Y separate toward opposite poles they quickly lose their club-shaped appearance and become more or less uniformly elongated.

To recapitulate: Of the two types of chromatin that compose both X and Y chromosomes each behaves in characteristic manner. The euchromatic sections follow the same procedure witnessed in the autosomes, and are subject to the same type of terminal attraction at diakinesis. A tetrad is formed by the two sex chromosomes entirely as a consequence of this behavior of the euchromatin, for the heteropycnotic ends behave like more orthodox sex chromosomes in showing no attraction for each other at this time.

The typical heteropycnotic attraction is, however, evinced at a stage before the diplotene. It is then that the heterochromatic regions usually come together and undergo fusion. It is during prophase also that the structure of this heteropycnotic chromatin merits some attention. For in gentian violet preparations it is then vacuolated and spongy—and instead of presenting the densely black mass seen in haematoxylin preparations, the heterochromatin may then actually be stained less intensely than the euchromatin (Figs. 1 to 7). It will be remembered that Bauer (1936) suggested that the shell-like chromomeres that characterize the heterochromatin in the sali-

1. Early diplotene stage showing the fused heteropycnotic sections of the sex chromosomes, the heterochromatin showing characteristic vacuolization.

2. Confused stage, still showing a single vacuolated heterochromatin nucleolus. Only the nucleus is represented.

3. Early diakinesis showing the split X and Y quite separate. The heterochromatin of the X is typically more elongated than that of the Y. Only the nucleus is shown.

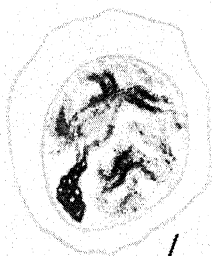
4 to 8. Progressive condensation of the sex chromosomes during diakinesis, with the terminal metaphase union taking place at the euchromatic ends. All drawings are placed so as to show the Y above the X.

9. Polar view of first metaphase. The X and Y at an obtuse angle to each other.

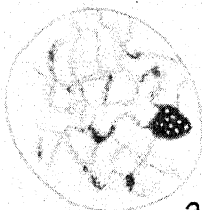
10 to 12. Successive positions of the chromatids of the X and Y during the first division. Y placed above the X.

13. Telophase of the first division, showing the clubbed (heterochromatic) ends of the sex chromosomes pointing toward the poles. Autosomes are not club-shaped. Chromatoid body on left side of lower cell.

14. Interphase cells, showing touch-and-go phase involving the clubbed ends of the sex chromosomes. In cell on left there is an achromatic connection between the X and Y. (X in both cells is above Y.)



1



2



3



4



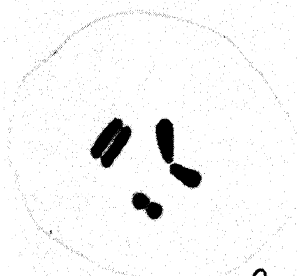
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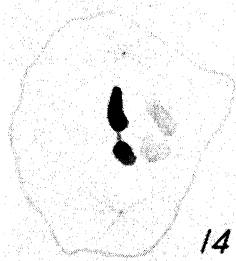
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14



13



vary cells of Chironomidae may also be encountered in the chromatin nucleoli of more orthodox cells. The vacuolated heterochromatin of *Rhytidolomia* indicates that this may indeed be the condition in its heteropycnotic chromatin. Therefore, if, as Bauer argues, heterochromatic attraction is in some way correlated with the structure of such chromomeres, the prophase union may be basically alike in these widely different cells.

The separation of the heteropycnotic ends of the sex chromosomes at or before diakinesis, and their reunion before the second metaphase, parallels the behavior of heterochromatin described earlier in a wide variety of cases. In *Rhytidolomia* the source of attraction seems to lie specifically in the clubbed or heterochromatic ends of the sex chromosomes, which would indicate that the touch-and-go reaction resides at least in part in the specific character of the chromatin and not alone in mitotic forces extraneous to the chromosomes. *Rhytidolomia* thus furnishes a natural demonstration of the difference in the meiotic behavior of the two forms of chromatin, and though it does not furnish stronger evidence for the special behavior of heterochromatin than the instances already cited, it is peculiarly striking in presenting a demonstration within a single pair of chromosomes.

## CONCLUSION

The evidence from a wide range of cases strongly indicates that during a certain period of the meiotic prophase there is a definite and specific tendency for all heterochromatin to come together. The strength of this attraction may well be affected by variation in the character of the heterochromatin. Although it is natural to homologize such an attraction with the meiotic pairing of euchromosomes, an analysis of the process points strongly to the contrary.

The touch-and-go phenomenon of sex chromosomes in the second division of Heteroptera is not so clearly a demonstration of such attraction. But though it is likely that it may involve other factors as well, the evidence furnished by *Rhytidolomia senilis* would argue that the heterochromatin plays at least a part in the approach of the sex chromosomes to each other at this time also.

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BICENTENNIAL CONFERENCE

## Chromosomal Interchanges

By

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IN THIS symposium in celebration of the bicentennial of the University of Pennsylvania it seems fitting to consider the historical relationships of our subject. Two hundred years is not a long time so far as the recorded history of mankind is concerned; it is a long time, however, in the history of biological research and especially of research in the subject of our symposium. In 1740 Benjamin Franklin and the other founding fathers of the University knew little about biology for the simple reason that biology at that time was an almost undeveloped subject. An adequate system of naming plants and animals was lacking at the date we celebrate, since it was only thirteen years later, in 1753, that Linnæus published his *Species Plantarum* and introduced to the world the binomial system of classification which has been used ever since in both botany and zoölogy. Benjamin Franklin and his colleagues, moreover, would not have understood any of the titles of this afternoon's program. The term *chromosome* was coined 140 years after the founding of the University, *germ plasm* 143 years after this date, and the word *Drosophila*, meaning "lover of wetness," has only been known since 1823 although it has been revered by geneticists since the first decade of the present century. The word *evolution* in the sense in which Darwin used it in his *Origin of Species*, published in 1859, could have had little meaning to Founder Franklin. The same is true of the word *genetics*, which was coined by Bateson in 1906, and of the word *cytogenetics*, which has come into general use only within the last few years.

A couple of years ago at the Richmond meeting of the A<sub>3</sub>S there was celebrated the one-hundredth anniversary of the cell theory. Something of course was known about cells before Schleiden and Schwann, but it is convenient to think of the cell theory as having been an active influence in biological

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thought for only about a single century. The newness of our subject is evident when we realize that two-thirds of the period of study of cells since Schleiden and Schwann is covered by the life span of the last speaker on our program, who has taken a leading part in the development of cytology and who has helped to make the zoölogical laboratories at Pennsylvania an outstanding center for the study of chromosomes. Chromosomes were discovered in 1873, when McClung was three years old. They were given the name of chromosomes in 1888 about the time when he was an eighteen-year-old freshman in Kansas University.

How discoveries come to be made has always interested me. On the present occasion it seems appropriate to say something about the history of the discovery of segmental interchange. Perhaps the most important date in the history is 1914 when Belling published his findings on semi-sterility in the velvet bean—*Stizolobium*. His account was a detailed and masterly analysis, and *Stizolobium* became the classical example of semi-sterility. He showed that in the  $F_1$ 's from crosses between certain forms 50 per cent of the pollen grains and egg cells are aborted, and concluded that semi-sterility has an hereditary basis. The problem of *Stizolobium* undoubtedly kept recurring to Belling and aided his thinking when he later worked with *Datura*. I remember his showing me pollen of a variety of *Rhododendron* in which two grains of each tetrad were aborted. Apparently the work with *Stizolobium* had suggested to him the study of pollen of Ericaceous species in which the four grains of a tetrad fail to separate, and he had chosen horticultural *Rhododendrons* because of their hybrid origin.

The next important date in our history is 1920, when Belling came to the Department of Genetics as a guest investigator. We were fortunate in securing his coöperation in a study of the chromosomes of the mutant types which had turned up in our cultures of *Datura*. By the use of the old acetocarmine method, which he greatly improved through the addition of iron, Belling made smears of pollen mother cells of the Globe mutant and reported the presence of an extra chromosome. There were several other mutant types which were inherited in much the same manner as was the Globe. One of these, named "Poinsettia," we had found to transmit the genes for purple (P), and white (p) flowers in very different proportions from the familiar



Mendelian ratios. It became apparent that these abnormal ratios agreed closely with what would be expected if the extra chromosome carried the locus for purple and white genes. The Poinsettia type gave us our first trisomic ratios.

Trisomic ratios have helped in proving the specificity of the extra chromosomes and have become the preferred method in *Datura* and maize for locating genes in particular chromosomes. In our present terminology, in which ends of chromosomes are numbered, the Poinsettia type is designated as  $2n + 17\cdot18$ . The white race which furnished the p gene for our first trisomic ratio came from the same bag of seeds, sent us from Washington, D. C., which furnished our purple standard Line 1. Later the  $2n + 17\cdot18$  type was crossed with another white race from Germany. It was not long before very wide departures from the expected trisomic ratios of purples to whites occurred in these later cultures. They occurred in the second backcross generation from individual trisomic parents which had the formula  $Pp_2$ . Instead of the ratio  $1P:2p$  following a male backcross, for example, the ratio became  $1P:6p$  or showed even a greater excess of whites. It was soon possible to prove that the trouble in the ratios lay with the German chromosome. Other white lines were tested. Some gave normal trisomic ratios like the white from Washington and were called "A" whites; others, like the white from Germany, gave an excess of whites from certain of the trisomic parents in the second generation and were called "B" whites. The classification into A's and B's was a tedious process since it was only a part of the trisomic parents in the second generation which could be used as testers. Later two speedier methods were discovered for separating B's from A's. In attempting to locate certain genes from nature by backcrossing the different  $2n + 1$  types, we found we had included both A and B whites. To our surprise we discovered that, among the trisomics heterozygous for B white, both  $2n + 17\cdot18$  and  $2n + 1\cdot2$  parents threw what seemed to be trisomic ratios, whereas when the  $2n + 1\cdot2$  parents were heterozygous for A whites they threw only disomic ratios. It was thus established that the B whites in some way involved both the  $17\cdot18$  and the  $1\cdot2$  chromosomes. Heterozygous  $2n + 1\cdot2$  parents were not used as testers for A and B whites since about this time a better tester was found in our mutant type called Nubbin, which, when heterozygous for B whites, gave in a backcross five

or more times as many whites as purples, but which gave a 1:1 ratio when heterozygous for A whites. Nubbin is a "compensating type" which was secured by radiation treatment in 1921. We now know its constitution to be  $2n - 1.2 + 1.9 + 2.5$ , but at the time when we began to use it as a tester for B whites we had not worked out its formula although we knew that in some way it involved the 1.2 chromosome. The method was relatively inexpensive. Male backcrosses from Nubbin give only  $2n$  offspring. Purple-flowered plants have red stems, white-flowered plants have green stems; the color classes therefore could be recorded in the seedlings and the seed pans discarded at an early stage.

By the fall of 1925, when Belling proposed his hypothesis of segmental interchange, we had been able by means of the Nubbin method to list eight of our white lines as B's and the same number as A's. Later by the same method we found that 98 of our white lines were B's and 31 A's. Later also we made up white Nubbins and, using them as testers, distinguished B from A purples in backcrosses from heterozygous Nubbins. By the Nubbin method we finally listed 17 of our purple races as B's and 25 as A's.

While the evidence regarding the nature of B races was accumulating from abnormal ratios of purples and whites, two mutant types with single extra chromosomes appeared from time to time in our cultures, and tabulations showed they had arisen only from parents which were heterozygous for B races. They were related to the  $2n + 17.18$  and the  $2n + 1.2$  types both in appearance and in breeding behavior. One called "Wiry" (now known to be  $2n + 1.18$ ) resembled the  $2n + 1.2$  and especially its secondary  $2n + 1.1$  from its erect habit, narrow leaves, and small stigma. It resembled the  $2n + 17.18$  from the peculiar adventitious outgrowths of tissue produced on its branches. Since the secondary  $2n + 17.17$  type does not produce this adventitious tissue, the peculiarity was supposed to be due to excess material from the .18 half. Like the  $2n + 17.18$ , Wiry gave trisomic ratios for purple and white. It also showed its relationship to these same primary trisomics by throwing in its offspring a significant percentage of both  $2n + 1.2$  and  $2n + 17.18$  types.

The second trisomic mutant which came from parents heterozygous for A and B races resembled in its drooping habit of

growth the  $2n + 17\cdot17$  secondary, which we had called "Dwarf," and in its sugarloaf-shaped capsules and large stigmas it resembled the  $2n + 2\cdot2$  secondary which we had called "Sugarloaf." From its appearance we called the type Dwarf-Sugarloaf. This name shows we had correctly recognized the effects produced by the extra chromosomal material which was present, since the name is only another way of telling the same story, so far as the appearance is concerned, as the present formula for the type which is  $2n + 2\cdot17$ . In breeding behavior the Dwarf-Sugarloaf, like Wiry, showed its relationships to the  $1\cdot2$  and the  $17\cdot18$  chromosomes by throwing both primaries  $2n + 1\cdot2$  and  $2n + 17\cdot18$ , in its offspring. It did not, however, give trisomic ratios for purple and white.

These are the genetic data which we presented to Belling with the suggestion that cytological study of Wiry might furnish the clew to the problem of the B and A races which had been found to occur in nature. Few could have attacked the problem better prepared than Belling. His work on semi-sterility in *Stizolobium* had given him the concept of forms differing in cryptic characters which produce a visible effect through interaction after hybridization. He had been the first to realize fully that it is likeness of the ends of chromosomes which causes them to be attached to each other in pairs at metaphase of the first meiotic division. Belling showed, for example, that the two secondaries which we now know as  $2n + 1\cdot1$  and  $2n + 2\cdot2$  may have closed circles of three chromosomes at meiosis, whereas the chromosomes of their primary  $2n + 1\cdot2$  can never form a closed configuration. By matching up the ends he correctly interpreted these chromosome attachments as proving that in secondaries one member of the trisome must be a double half chromosome.

In the trisomic Wiry, Belling found a small chromosome similar in size to  $17\cdot18$  attached to a group of two large chromosomes of the  $1\cdot2$  size. The chromosomes were first designated by numbered ends in a joint paper with Cleland several years later, but will be used here for convenience. Following his successful method of interpreting the chromosome attachments in secondaries, Belling concluded that one of the three attached chromosomes in Wiry must have an end from a non-homologous chromosome. Such a chromosome has since been called a tertiary chromosome, and the type with it as an extra has been called a

tertiary  $2n + 1$ . The hypothesis fully explained our B races, the 1-18 and 2-17 chromosomes of which were considered to have originated by interchange of segments of the non-homologous chromosomes 1-2 and 17-18.

The experience with *Stizolobium* apparently led Belling into a minor error in that he believed plants of *Datura* which were heterozygous for the B complex should be semi-sterile. Since we had a considerable collection of races containing both B's and A's which had been intercrossed in a study of distribution of color factors, the character of the pollen was soon tested. In hybrids between B races and our standard Line 1 the pollen was good; however, in hybrids with our Line 26, which is an A white, the pollen had 50 per cent aborted grains. Further tests showed that this Line 26 was a "bad pollen inducer" in that hybrids between it and most of the other lines were semi-sterile. We now know that this exceptional line belongs to Prime Type 7 which always induces semi-sterility in hybrids with other chromosomal races.

By using a Prime Type 7 race as a tester in crosses with all our other lines from nature we were able to classify these races as belonging to PT 7 type if the pollen in the hybrid was good and as being different from PT 7 if the pollen was 50 per cent bad.

At this time Belling was no longer at Cold Spring Harbor. A closed configuration of 4 attached chromosomes was found in heterozygous B plants through the help of Rachel Haynes and Louise Buck, and this finding was confirmed by Belling from preparations sent him. Later, with the cytological help of Dr. Bergner, we undertook a study of chromosome interchanges by the presence or absence of configurations in hybrids between our standard Line 1 tester of *D. stramonium* and over 600 races of this species from nature. Including this standard tester, which we call Prime Type 1 and in terms of which we classify the other types, we have found only 5 commonly recurring chromosomal types in nature. Prime Type 2 (our old B race) has the interchanged chromosomes 1-18 and 2-17, PT 3 has the chromosomes 11-21 and 12-22, PT 4 has chromosomes 3-21 and 4-22, and PT 7 has chromosomes 9-10<sup>20</sup> and 19-20<sup>10</sup> in which the terminal satellites only are interchanged. The United States has a mixture of types with PT 1 predominating. PT 1 is the only type found in Brazil. PT 2 is practically the exclusive type in Asia.

PT 3 in combination with PT 2 is the only type found in Peru and among the purple forms in Chile. Western Europe has a mixture of types. The isolated types which have been turned up number only four. It is evident that in *Datura stramonium* there are only a limited number of chromosome types which have become established through segmental interchange.

Spontaneous interchanges have also been uncommon in our controlled cultures. In all our experience we have had the spontaneous occurrence of only three tertiary  $2n + 1$  types which presumably came from previous chromosome interchange. The tertiary  $2n + 4,6$ , for example, presupposes a previous interchange between the 3,4 and 5,6 chromosomes. To test the possibility that interchanges which had arisen earlier might have remained hidden in inbred stock, Dr. Bergner studied cytologically the  $F_1$  hybrid between our Line 1 and twenty-four of the primary and secondary lines some of which had been kept going by selfing for as many as ten generations. She found no evidence for any interchanged chromosomes; the chromosomes of *D. stramonium* appear therefore to be relatively stable in constitution.

Standard lines have been established in all of the other 9 herbaceous species in *Datura* and used as chromosomal testers. In all the species in which an adequate number of races has been available, Prime Types with interchanged chromosomes have been discovered. In such species the number of Prime Types is limited as was the case with *D. stramonium*. By hybrids with the tester races within each species we are attempting to discover what are the end arrangements of their chromosomes in terms of the chromosomes of our standard Line 1 in *D. stramonium*. By this method we have so far determined the chromosome ends in *D. stramonium*, *D. ferox*, *D. quercifolia*, *D. discolor*, and *D. Leichhardtii*. Only the two chromosomes 5,6 and 13,14 appear to have the same end arrangements throughout these 5 species; the other 10 chromosomes have undergone from 1 to 8 interchanges in the origin of the different species or their chromosomal varieties. When a larger number of species and their chromosomal types are analyzed it will likely be found that there is none of the chromosomes in the genus which has not undergone segmental interchange.

Chromosomal interchange has brought about a redistribution of genes in chromosomes. It cannot be shown, however, to have

been responsible for the origin of species although it appears to have accompanied their evolution. There are no species of *Datura* known which fail to show configurations in the  $F_1$  with other species, a fact which indicates that segmental interchange occurred in the origin of some of their chromosomes. The taxonomic distinctness of two forms, however, cannot be related to the number of such interchanged chromosomes. Thus if any one of several of the races of *D. stramonium* from the Barbados (which are PT 4 and 7) are crossed with any of the races of the same species from Peru (which are Pt 2 and 3), the  $F_1$  would have two circles of 4 chromosomes and 1 circle of 6, indicating a difference between these forms in end arrangements of 14 of their 24 chromosomes. If, on the other hand, hybrids were obtained between a race of *D. stramonium* from Hungary (which would be PT 2) and the standard race of *D. Leichhardtii* (which belongs to a separate group of the genus *Datura*) there would be only a single circle of 4 chromosomes in the  $F_1$ . This would indicate a difference between these very distinct species in end arrangement of only 4 chromosomes. Similarity of chromosome ends may be of value in attempts to unravel phylogenetic tangles. As we have earlier pointed out, the chromosomes of *D. ferox*, *D. quercifolia*, and *D. discolor* have end arrangements more similar to the Peruvian than to any of the other known types of *D. stramonium*. The overlapping distribution of these forms as well as certain genetic characters indicates that their evolutionary relationships with *D. stramonium* are through the chromosomal races of this species which are found in Peru.

It should be emphasized that the contribution of the *Datura* investigations to evolution of chromosomes has to do almost entirely with shifts of chromosome ends through segmental interchange. There is genetic and cytological evidence, however, that changes in blocks of genes have taken place inside the chromosomes. For example, Dr. Bergner has identified chromosome bridges which are indications of inversions. Chromosome evolution in *Drosophila* through inversions has been demonstrated by Dr. Dobzhansky. It is an open question why races of *Drosophila* show an abundance of inversions but give no evidence of segmental interchange which is readily demonstrated in *Datura*.

We have discussed chromosomal interchanges in nature. In the laboratory they have been induced in abundance by treat-

ment with radium, X-rays, heat, and aging of reproductive cells. Of these we have 85 numbered Prime Types which we have rendered homozygous for the interchanged chromosomes in addition to the Prime Types from nature. Of the induced types, 59 were due to segmental interchange (reciprocal translocation). Forty-nine were capable of giving closed circles of 4 chromosomes (46 cases) or 6 chromosomes (3 cases) when heterozygous. Ten were due to segmental interchange which involved small terminal portions (generally satellites) of the chromosomes and gave configurations which we have called neckties since they generally show a collar-like pair of chromosomes attached to a smaller often open pair by means of the connecting satellites or terminal humps. There were 15 cases of what we have interpreted as simple translocations since they induce open chains of chromosomes, never closed circles. It seems strange that no simple translocations leading to non-closable chains when heterozygous have been found in nature. Judging from the number of modified chromosomes which have come from simple translocations induced by treatments, we should have expected more than 6 chromosomes from among the 39 modified chromosomes observed in 7 species.

All the necktie inducers (the PT's with interchanged humps) give 50 per cent aborted pollen grains and egg cells but none of the closed-circle formers do so. Of the simple translocations 10 induced 50 per cent abortion, 3 induced 25 per cent, and 2 had good pollen when heterozygous.

Of especial interest are the interchanges which form closed circles in hybrids with the standard tester. In plants which have a "B" circle, for example, the pollen and egg cells are as good as in pure Line 1 normals—a fact which confirms the observation that the chromosomes at meiosis are arranged on the spindle in such a way that adjacent members of the circle go to opposite poles. Since with this type of disjunction only the parental types appear in the gametes, semi-sterility is not expected and does not occur.

Some plants with closed circles may have around 25 per cent or less aborted pollen. This is true for heterozygous PT<sub>3</sub>. The PT<sub>3</sub> circle frequently breaks prematurely into chains of two, the disjunction from which would lead in half the cases to deficiencies and hence to aborted pollen grains. Heterozygous PT<sub>3</sub> plants when grown in the field regularly have very close to

25 per cent aborted pollen, but when the plants are grown in the greenhouse in winter the percentage of bad grains may run up to nearly 50 per cent or down to nearly 0, suggesting influence of environmental factors on chromosome associations at meiosis. Of the circle formers in *Datura*, 35 gave practically good pollen, 11 gave 25 per cent aborted grains, and 4 gave a distinct percentage but not over 15 per cent of abortion.

In maize the condition is quite different. In most cases heterozygous Prime Types have been picked out by the 50 per cent bad pollen and the circle later identified cytologically. A collection of Prime Types made in this way therefore is not a random sample and fails to give evidence regarding the frequency with which 50 per cent bad pollen accompanies circles in maize. The only random sample in maize with which we are familiar is a collection of around 25 Prime Types made by Stadler in which the configuration was discovered first and the amount of aborted pollen determined later. In all these cases, which were personally communicated to us by Dr. Stadler, the circles were associated with semi-sterility. It seems to be the rule that in circles in maize adjacent chromosomes go to the same pole with great frequency while in *Datura* they go to opposite poles perhaps invariably. Darlington attributes the difference to the fact that in *Datura*, where adjacent chromosomes go to opposite poles, the terminalization is usually complete, while in maize, in which adjacent chromosomes go to the same pole, terminalization may or may not be complete. However, the differences between *Datura* and maize in chromosome behavior at meiosis in relation to gametophytic sterility would seem to need further study.

It will not be possible in the time available to discuss interchanged chromosomes in other forms. Dr. Cleland, who speaks next, is the authority in best position to explain how interchanges have guided evolution in *Oenothera*, that classical genus on which de Vries founded his mutation theory. Time also prevents our explaining how interchanged chromosomes may be used in synthesizing artificial new species.

Interchanges are only one of the types of chromosome alteration which have evolutionary significance. Our contribution therefore is only a partial view of a larger picture. The key to the problems of organic evolution we believe is to be found in a study of all aspects of the evolution of chromosomes.



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Chromosomal Differences Between Races  
and Species of *Drosophila*

*By*

TH. DOBZHANSKY\*

THE historic development of the organic world may be thought of as occurring in two different ways. The origin of new forms of life may take place gradually, so that important departures in the make-up of organisms are brought about only by integration of small individual steps; or else, new kinds of life may spring up suddenly, owing to cataclysmic upheavals in the germ plasm of their ancestors. Theories postulating the former of these two methods of change are spoken of as evolutionary ones, since according to them the living matter develops, unfolds, i.e., evolves. Such are the theories of Darwin and of most of his predecessors and followers. Theories of cataclysmic origin of new forms assume no evolution but revolution and catastrophes. Here belongs the theory of creation, some of the transformistic notions of the ancients, the views of G. St. Hillaire, and perhaps those of de Vries. In a recently published book Goldschmidt develops the first catastrophic theory supposedly based on genetic data.

The distinction between evolutionism and catastrophism is methodologically simple enough. If species arise by cataclysms, the living world must be divisible into rigidly discrete units separated by unbridgeable gaps. Since a biological system based on recognition of perfectly discrete units would be, for pragmatic reasons, an ideal one, taxonomists have endeavored for two hundred years to discover such units. They found species to be the most "natural" category, but they failed to prove the universality of unbridgeable gaps between species or any other groups. It is this signal failure that constitutes the basis of all evolution theories from Lamarck and Darwin to our day. If the organic world has evolved, the gaps between the existing species must be due to rarity or disappearance of the intermediates.

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Hence, one must be able to find examples of species in the making, and to demonstrate that any kind of difference distinguishing species may sometimes be present, at least as a vestige, within species.

A discussion of the whole problem of evolutionism *versus* catastrophism being here out of the question, I restrict myself to only one of its aspects, namely that concerning the evolution of the chromosomal apparatus. The early genetic and cytological literature tended, on the whole, to emphasize chromosomal differences between species and a uniformity within a species. The rules of the constancy of chromosome numbers and shapes, and of the constancy of the gene arrangement within the chromosomes, are among the important tenets of genetic and cytological theory. To be sure, modifications of chromosomes were observed very early. Such were the variations in the number of chromosome sets (polyploidy) and of individual chromosomes (polysomy), which were found to arise as abrupt changes. Later came "mutations" affecting the gene arrangement in the chromosomes—deficiencies, duplications, translocations, and inversions. But, with few exceptions, these mutations were laboratory products, and little was known regarding their occurrence in natural populations. Still more recently the situation has, however, changed. At present it appears that every known kind of chromosomal difference between species can be observed in nature within a species as well. This point is well illustrated by observations on representatives of the fly genera *Drosophila* and *Sciara*.

In *Drosophila pseudoobscura* twenty-one gene arrangements in the third chromosome, six in the second, two in the fourth, and three and two in the right and the left limbs of the X-chromosome respectively have been detected in natural populations. All of these arrangements could be, and presumably were, derived from each other by inversion of segments of chromosomes. The variations in the gene arrangement show the same phenomena that are familiar to taxonomists working with visible external characteristics of animal and plant species. *Drosophila pseudoobscura* is differentiated into geographic races, each characterized by relative frequencies of the various gene arrangements in the populations. Every gene arrangement has a definite geographic distribution, some being widespread and others endemic, but no arrangement occurs throughout the species

area. The population is seldom, if ever, uniform in any given locality, and frequently as many as six arrangements coexist side by side. Individuals homo- and heterozygous for gene arrangements are found, and the observed frequencies of hetero- and homozygotes are in accord with calculations based on the assumption of random mating.

In passing from one part of the distribution area to another the changes in the composition of the population are, as a rule, gradual, so that geographic gradients or clines are observed. The following table shows the frequencies of some of the gene arrangements in the third chromosome (in percent) in a series of localities in the southwestern United States, the localities being arranged roughly from West to East.

Locality	Gene arrangement				Chromosomes examined
	Standard	Arrow-head	Chiricahua	Pike's Peak	
San Rafael Mts., Calif.....	52.2	21.7	13.0	—	92
San Jacinto Mts., Calif.....	39.6	28.9	27.9	—	4329
Western Death Valley region, Calif.....	30.6	51.6	15.8	—	2834
Eastern Death Valley region, Calif. & Nevada.....	17.4	71.1	11.5	—	1194
Prescott, Arizona.....	11.0	79.0	9.0	1.0	100
Grand Canyon, Arizona.....	0.5	97.0	2.5	—	200
Flagstaff, Arizona.....	1.0	97.0	1.0	1.0	100
Mesa Verde, Colorado.....	—	100.0	—	—	100
Raton Pass, New Mexico.....	—	80.0	0.9	18.2	110
Trans-Pecos Area, Texas.....	1.4	35.9	3.5	53.5	142
North-Central Texas.....	—	21.5	—	70.2	1315
South-Central Texas.....	0.2	11.7	—	70.3	418
Valley Area, Texas.....	—	3.3	—	76.7	30

It may be seen that the farther two populations are removed geographically the greater the differences in their composition tend to be. Thus, populations from southern California are qualitatively similar, although significant quantitative differences are observed between them. The same holds for Arizonan and Texan populations. Populations of California and Texas are, however, sharply distinct: with the exception of Arrow-head, all the gene arrangements that are common in California

do not occur at all or occur very rarely in Texas, and vice versa. The populations residing farther south, in Mexico and Guatemala, are more sharply different from the Californian and Texan ones than these are from each other. Finally, the populations of Mexico and Guatemala on one hand and those of Washington and British Columbia on the other have so little in common that only occasionally is the same gene arrangement encountered in both.

Of course, no exact proportionality between distances and racial differences is either observed or expected. The portion of North America where *Drosophila pseudoobscura* is indigenous, is physiographically very complex and has had an involved geological history. Moreover, the regularity of the geographic gradients diminishes if population samples come from localities the distances between which are very small. In localities fifty or less miles apart the populations are frequently distinguishable, but the variations seem to be, in the geographic sense, haphazard. In addition, repeated sampling of the population in the same locality frequently shows that its genetic composition does not remain static. In fact, these micro-racial units appear to be in a constant flux: the relative frequencies of the gene arrangements undergo statistically significant changes not only from year to year but often from month to month. Although such elementary evolutionary changes, observable well within a human lifetime, are a fascinating subject for study, this topic is outside the scope of the present article. What is important for us now is that all stages of racial differentiation, from a slight difference discernible only by means of statistical analysis and up to a clear qualitative distinction, can be found in the chromosome structures of a single species, *Drosophila pseudoobscura*.

Chromosomal variations are known also in species of *Drosophila* other than *D. pseudoobscura*. Although the situation is rather similar in all of them, certain suggestive differences have been recorded. As pointed out above, in *D. pseudoobscura* the variations in the gene arrangement affect chiefly the third chromosome, the other chromosomes being relatively stable. The same appears to be true in *D. miranda*, a close relative of *D. pseudoobscura* (Koller). Yet, in species of the *D. affinis* group (*D. azteca*, according to Dobzhansky and Socolov, *D. algonquin*, according to Miller, *D. athabasca*, according to Novitski), in *D. melanogaster* (Sturtevant, Dubinin, and collaborators), and in

*D. ananassae* (Kaufmann, Kikkawa) several chromosomes are variable. What are the causes of such differences in variability of different chromosomes in natural populations is unknown. Bauer, Demerec, and Kaufmann found that in *D. melanogaster* the breakages induced by X-ray are distributed among the chromosomes at random, i.e., in proportion to their lengths. The same result was obtained by Helfer in *D. pseudoobscura*, although here the third chromosome could be expected to be more breakable than the rest.

It is important to note that in comparing the chromosomes of related species the greatest differences in the gene arrangement are usually found in the same chromosomes which show the greatest variability within these species. Thus, in *Drosophila pseudoobscura* and *D. miranda* the third chromosomes are built very differently, while some other chromosomes (fifth, second, XR) are more similar. In *D. azteca*, *D. algonquin*, and *D. athabasca* the C and D chromosomes are relatively constant, while A, B, and X-chromosomes are changed almost beyond recognition. Another difference in the chromosomal variability of different species deserves a brief mention. In *D. melanogaster*, and apparently also in *D. ananassae*, there is a normal or standard gene arrangement in all chromosomes, which occurs throughout the studied parts of the geographic areas of these species. In *D. pseudoobscura* and other species the racial differentiation has progressed so far that no gene arrangement can be described as normal for the species as a whole. This may mean that some species are older than others, or inhabit territories with a topography and history more conducive toward divergent evolution. On the other hand, species like *D. melanogaster* and *D. ananassae* are largely scavengers associated with man's economy, and in most countries their distribution is either very recent or greatly modified.

We may now consider the chromosomal differences which exist between species as such. Although the available data are meager, examples of various degrees of divergence may be cited. According to Tan and Koller, the chromosomes of race A and race B of *Drosophila pseudoobscura* differ in four inversions in the two limbs of the X, the second and the third chromosomes respectively. More recent observations have shown that the gene arrangement characteristic for the right limb of the X-chromosome of race A is encountered occasionally in race B as well; as

to the third chromosome, 13 arrangements are known which occur only in race A, 7 are restricted to B, and 1 arrangement is encountered in both A and B. Thus, only two inversions, one in XL and one in the second chromosome, differentiate the races. It must be noted that these "races," although scarcely distinguishable morphologically, show partial sexual and ecological isolations, and produce upon crossing sterile male and semi-fertile female hybrids. In other words, we are dealing here with closely related species rather than with races. The chromosomal differences between them are smaller than those which may be encountered between strains of the same species. *D. virilis virilis* and *D. virilis americana* have been described as subspecies (Spencer). According to Hughes, at least five inversions, distributed among four out of the six chromosome limbs present in species, may be seen in the *virilis*  $\times$  *americana* hybrids. Furthermore, four of the rod-shaped chromosomes of *virilis* are represented in *americana* by two V-shaped ones, indicating that translocations have taken place in the phylogeny. This aspect of the situation will be discussed further.

The gene arrangements in *Drosophila pseudoobscura* and *D. miranda*, which by any criterion must be classed as distinct species, differ very widely. At least ten inversions and no less than three translocations must have taken place in the phylogeny. The inversions involve mostly long, and the translocations relatively short, chromosome sections. Moreover, the homology of many sections is unclear, since the disc patterns observed in them in one species cannot be detected anywhere in the other. On the whole, the architecture of the chromosomes of *D. pseudoobscura* and *D. miranda* is so different, and their pairing in the hybrids is so greatly disturbed, that the homologies can be unraveled only with difficulty (Dobzhansky and Tan). Among pairs of species of *Drosophila* the hybrids between which can be obtained, *D. azteca* and *D. athabasca* have most widely different gene arrangements (Bauer and Dobzhansky). Using the criterion of chromosome pairing in the salivary gland cells of the hybrids, it has been possible to establish the homology of only a few chromosome sections. Two chromosome limbs (the D chromosome and a limb of C chromosome) are built very similarly in both species. Elsewhere, several inversions have undoubtedly taken place, but more than half of the chromatin mass in either species seems to have no homologues in the other.

In species which cannot be crossed, the only remaining cytological criterion of chromosome homology is the similarity of the disc patterns in the salivary gland chromosomes. The information obtained is almost of necessity fragmentary, but it is nevertheless possible to infer that rearrangements of the genic materials take place in the phylogeny on a very large scale. Species so close as *Drosophila melanogaster*, *D. pseudoobscura* and *D. azteca* (belonging, according to Sturtevant, to the same subgenus) show no recognizably identical chromosome sections whatever. Some curious, and perhaps misleading, similarities have, however, been observed. The free end of one of the autosomes in *D. subobscura* (a species related to *D. pseudoobscura* and *D. miranda*) appears to be similar to the free end of one of the limbs of the X-chromosome in *D. azteca*. It is possible that the inversion characterizing the so-called sex-ratio condition in race A of *D. pseudoobscura* causes the tip of the X-chromosome to resemble those in *D. azteca* and *D. subobscura*.

The presence of chromosome sections in one species which seem to have no homologues even in rather closely related species naturally demands an explanation. There can be little doubt that a majority of such cases are due to changes in the gene arrangement by successive inversions which eventually alter the gene arrangement beyond recognition. On the other hand, facts are accumulating which suggest that processes other than gene rearrangements may modify the cytologically visible properties of the chromosomes. In the hybrids of *Drosophila melanogaster* and *D. simulans* several short sections in various chromosomes fail to pair, although only a single major inversion is present in one of the chromosomes (Patau, Kerkis, Horton). A careful examination of these sections reveals that they consist of discs which have a general resemblance in the two species, and yet are slightly different in their staining properties and appearance. Similar minor differences have been observed in *D. pseudoobscura*  $\times$  *D. miranda* hybrids, alongside with the major inversions and translocations mentioned above (Dobzhansky and Tan).

It is, of course, possible that these minor differences in the chromosome makeup are due to inversions or translocations of very small chromosome fragments. On the other hand, it is conceivable that processes akin to gene mutation may alter both

the cytological appearance and the pairing properties of chromosome sections. This point is important because no comparable changes have as yet been established within species of *Drosophila*. The situation is, however, very different in the fly genus *Sciara* studied by Metz and his collaborators. The salivary gland chromosomes of the hybrids of *S. ocellaris* and *S. reynoldsi* show no gross differences ascribable to major inversions and translocations. Nevertheless, the chromosome pairing is disturbed, and a careful comparison of the chromosomes section by section reveals numerous minor differences in the conformation of single discs or small groups of discs. As a counterpart to this situation in the species hybrid, Metz finds that strains of the same species of *Sciara* not uncommonly differ in the appearance of certain discs here and there in the chromosomes. On the other hand, major inversions, which are so ubiquitous in species of *Drosophila*, are at least rare in *Sciara*.

Where species cannot be hybridized, and where an inspection of the disc patterns in salivary gland chromosomes fails to detect identical chromosome sections, the gene arrangements can be compared only through the genetic chromosome maps. It is well known that similar mutants appear in species of *Drosophila* belonging to the same as well as to the different subgenera; the locations of the genes giving rise to these mutants are determined by their linkage relations. The pitfalls of this method are obvious: an apparent similarity of mutant phenotypes does not prove the identity of the genes producing them. Numerous examples of distinct genes lying in the same or in different chromosomes giving rise to mimetic mutants are known in several species. Nevertheless, this method has proved its usefulness in the hands of Sturtevant, Donald, Tan, and others. According to these authors, phylogenetic changes in the gene arrangement in the genus *Drosophila* take place chiefly through inversions within a chromosome limb; inversions involving the spindle attachments do occur, but are much less frequent than the interstitial ones; translocations uniting or separating whole chromosome limbs are recorded in several sections of the genus; translocations with breaks within the chromosome limbs have not been encountered.

It is easy to see how closely the results of comparison of different species parallel the observations on the intra-specific chromosome variability. Interstitial inversions are by far the



commonest changes detected in natural populations. Inversions including the spindle attachments are rare, but Miller has observed such an inversion in *Drosophila algonquin* and Dobzhansky in *D. duncani*. Only translocations, whether of parts or of whole chromosome limbs, have not been found in nature in species of *Drosophila* (except in the case of *D. virilis virilis* and *D. virilis americana*, which may, however, be regarded as separate species). The absence, or at any rate scarcity, of translocations in nature is theoretically understandable. Individuals heterozygous for translocations, and to a lesser extent those heterozygous for inversions including spindle attachments, produce many gametes with unbalanced chromosome complements. In turn, such gametes usually give rise to inviable zygotes. Heterozygosis for interstitial inversions does not, except in special cases, lead to comparable results. The reproductive potential of translocation heterozygotes is, therefore, lowered. Since translocations, as any mutant changes in the broad sense of this term, arise among masses of normal individuals, they are subject to adverse selection and are more or less promptly eliminated.

The objection may be raised that the above reasoning proves too much, since translocations have taken place in the phylogeny of *Drosophila*, and since some translocations have been encountered within a species in some animals (e.g., in *Orthoptera*, observations of Carothers and of White). The answer is that under certain exceptional conditions translocations and inversions involving the spindle attachment may become not only established in natural population but may play an important rôle in speciation. In the first place, if these chromosomal aberrations produce, through position effects, changes that are favorable to the viability of their carriers, the advantages so incurred may counterbalance the lowering of the reproductive potential due to the production of unbalanced gametes. Secondly, the unfavorable effects of translocations and of inversions involving the spindle attachment are restricted only to heterozygotes. Hence, as soon as a majority of individuals in a population becomes homozygous for a translocation or an inversion of this type, the selection pressure, which had tended to eliminate these changes when heterozygous, not only ceases to operate but actually becomes reversed in

sign (i.e., tends to eliminate the initial, or ancestral, chromosome structure).

The possibility that a translocation may, soon after it has first appeared, be present in a majority of individuals is, of course, practically nil in species with large undivided populations. The situation is, however, different in a species, even a widespread and common one, which is subdivided into more or less isolated colonies, the colonies having a small effective size or periodically becoming reduced to very small numbers. As pointed out by Wright, a translocation appearing in one of such colonies has a finite chance of becoming homozygous before it is swamped by adverse natural selection. Furthermore, once a colony is homozygous for a translocation, the interbreeding between it and the rest of the species retaining the original chromosome structure will lead to a lowering of the productivity of the hybrids. This may engender the development of isolating mechanisms which diminish the extent of the interbreeding, and finally eliminate it altogether. In such a way, the establishment of a translocation may initiate the splitting of an originally homogeneous species into two new ones. The fluctuations in the genetic constitution of local colonies with time (see above) attest that the precondition for the enactment of the above chain of events, namely a subdivision of the species into colonies with a small breeding size, appears to be present in at least some of the *Drosophila* species.

It seems justified to conclude that, with the exception of translocations, all the types of chromosomal changes which are known to differentiate species are met with in races as well. The rarity of translocations in natural populations is, however, not unexpected on theoretical grounds. Thus, one of the principal tenets of evolution theories, namely that under certain conditions races become incipient species, is confirmed by results of cytogenetic studies. Needless to say, a host of problems remain unsolved. One of the most important ones is what factors determine the spread and establishment of chromosomal changes, first as racial and then as species characters. In all likelihood, there can be no single cogent solution of this problem. Some gene rearrangements may, by position effects, produce physiological and morphological changes functionally equivalent to those produced by gene mutations. Their fate in natural populations will, accordingly, be governed princi-

pally by natural selection. Others have no discernible effects on their carriers, and hence appear to be neutral characters. Here we are dealing with a part and parcel of the problem of the evolutionary rôle of non-adaptive changes in general. As pointed out above for translocations, and as shown earlier by Sturtevant for certain combinations of inversions, chromosomal changes, even if ecologically neutral, may play a somewhat special rôle as initiators of species divergence.



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## Evolution of the Germplasm

*By*

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A DEFINITION of terms seems to be the first requisite in a discussion of this subject. By "germplasm" we mean a distinctive substance, endowed with all the properties of life, but especially with that of reproduction, which here is, in some measure, unique. Equivalence is an inherent characteristic of organic reproduction, and it holds in respect to the germplasm itself; but whereas commonly the influence of a part is continuously the same, that of the germplasm is cyclically different. It characterizes changes which mark the race and so it may be denominated "racial material." It is customary to distinguish between "germplasm" and "somaplast," both being nuclear; one concerned with racial processes, the other with those of the individual. This distinction is, however, purely arbitrary and may lead to misunderstandings. Such a distinction was suggested by the presumed functional differences between the macro- and micronuclei of certain Protozoa. But if the germplasm is defined specifically as "that substance, or organization which distinguishes a chromosome complex," then it is essentially the same in both germ and somatic cells. Mere observation tells us that the chromosome complement of germ and somatic cells is one, each being derived by direct descent from that of the original zygote. Finally, and in a more abstract sense, the germplasm may be defined as "the temporal record of racial experience." However conceived it has the properties of continuity, specificity, and control of organic processes.

The term "evolution" signifies a process characterized by progressive, continuous, and related change, as opposed to one in which sudden, discontinuous, or unrelated modifications occur. Broadly conceived, it includes not only the stages of full functioning but also the ones which may be characterized as

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formative. The germplasm did not always exist. Only when the earth reached a certain balance in temperature, moisture, radiation, and other physico-chemical conditions was life possible. Organic evolution is therefore clearly a part of cosmic evolution. All the evidence in our possession indicates that the first organic entities were small and relatively simple. Since probably the most significant attribute of life is that it exists only in unit form, our search for the beginning of organic things leads us necessarily to the conception of minute, simple units. That these may still occur as separate and independent bodies is suggested by the existence of such related organizations as the filterable viruses; that they persist as parts of co-ordinated aggregates follows from our conception of genes. The simplest of known organisms is relatively so complex that we cannot conceive it as coming into existence fully formed. We must believe that it is the result of a gradual development from small and simple beginnings which, by assumption, had the properties of life in essence. Therefore these were units in a continuous series, perpetuated by the inherent power of reproduction. The only continuous living thing we know is the germplasm, and we naturally associate it with these early beginnings. Thus conceived the germplasm of an existing animal has within it the direct descendants of successively added units, which have arisen in response to altered conditions, both within and without a series of organisms. Unit organization, perpetuated in a continuous series, addition of new elements in response to changed conditions, incorporation of these into a co-ordinated union of higher complexity, and finally the formation of the very complex structure we call a cell summarizes the series of events as we must now conceive them. In turn cells became aggregated into co-ordinated bodies of almost infinite complexity and variety. Originally the germplasm was, according to this view, in immediate relation with the environment. Gradually it was removed further and further from this physical contact until now only such agents as radiations may touch it directly. From the very first stage of its existence the germplasm has been directive in its relations. At first simple and applied directly to the materials and conditions of the environment, its influence has become more and more complex, involved and remote from the operations of the organism, particularly in the germ cells. Even further than we have thought,

this is true also of the somatic cells. During development more and more coördinating mechanisms, neural and humoral, are formed until finally it would seem that, for the nuclear materials, there remain only a few of the most basic functions. Each type of cell becomes highly specialized through modification of its cytosome, while its nucleus may lose its reproductive power or even disappear completely. For the individual there is, then, an exaltation of the cytosomic elements and a limitation of the nuclear. Only in the germ cells are nuclear potentialities retained. Retention of this power by the germ cells is made certain by various isolation devices which remove them from participation in differentiation during development.

But since the race is only a succession of individuals, how can we differentiate between activities which serve only the single individual and those which concern the group? Naturally this distinction cannot be closely drawn, and in fact it is largely temporal. A germ cell of one individual generation, isolated from somatic participation, becomes detached and forms a complete organism of the next generation. By some insulating device the germinal elements within the gonad do not participate in somatic processes, but merely perpetuate themselves. On being removed from this inhibition they are free from the limited rôle of mere germ cells and may perform, through their descendants, all somatic activities. The germplasm, the record of experiences to be repeated, is contained in all cells—in somatically included germ cells, there inhibited from full expression; in differentiated somatic cells, limited largely to a single expression; and is completely lost only in cells about to disappear. Distinctions between germ and somatic cells, unless early established by some marked change, as in *Ascaris*, may therefore be gradually established by specific limitations within somatic cells and by the retention of unaltered capacities in the germ cells. Fixation and limitation of function, it is assumed, is due to changes within the racial material through repeated reactions with a specialized cytosome.

But how are we to gain any practical knowledge of such changes as occur during differentiation and development? Surely only by a comprehensive and detailed study of the germinal material during these stages. Here we note that there is a general pattern in all Metazoa, which at once suggests a basic unity of design and function; also that there are char-

acteristic group modifications of such a character as to indicate that this is progressively increased in complexity in the phylogenetic series. Cytogenetic studies have demonstrated that the germplasm is a precise organization of particulate, causal elements which are specific in nature and yet general in their attributes. Which is perhaps to say that of a common series of functions, they show, individually, emphasis upon certain ones of these. Such studies have, however, yielded no knowledge of the method by which these causal elements operate. By limited and experimental investigations it may be possible to learn the individual operation of this mechanism, but knowledge of its phylogenetic development can come only from a careful comparison of its expressions in a group of organisms. But for this there is first required a careful analysis of the fundamental relations of organisms so that it is possible to evaluate the differences which characterize groups.

Every organism is a fully developed and coördinated unit. In this measure all organisms are alike. At the same time they differ, even individually. In estimating phylogenetic relationships what relative values have these elements of likeness and unlikeness? Since organisms are completely specific, structurally and functionally, at every stage of development, these qualities must exist even in the one-cell stage. When therefore we seek to discover, in the causal mechanism of the chromosomes, differential conditions between taxonomic groups what shall we look for? Obviously from the considerations just stated, great differences cannot obtain. We see indeed that they do not. Except for the lowest forms all organisms are composed of cells, and these are of a limited number of kinds wherever found. For each distinctive function there is a common form of appropriate cell. The visible differences between members of these types is in no way commensurate with the phylogenetic standing of the organisms in which they are found. High development and perfect coördination of structure and function occur at every taxonomic level. Since, then, individual structural units as such are no measure of phylogenetic advance, we must seek elsewhere for a criterion, and the course of individual development offers suggestions. There we witness, in addition to the differentiation of cell types, two suggestive phenomena, i.e., increase in the number of elements, and second in their interrelations, which mean greater complexity.



Such a situation carries with it the need for more extensive and perfect measures of control and correlation. This coördination is primarily provided by the nervous system, therefore in higher forms there is a more extensive development of this system. However, for purposes of regulating internal functioning, this control system does not anywhere differ widely. It is only when the level of ideation and reason is reached that a new problem presents itself, and we have to enquire whether human nerve cells develop new attributes, or whether higher functioning results from increased and bettered interrelations between them. Practically that is not a problem for those who seek an explanation of the relation between the casual mechanism of cells and developmental processes, for these do not immediately involve the subsequent phenomena of ideation. Our problem may accordingly be stated thus: In the presence of a vast series of structurally different types of organisms, all performing a common series of functions, where shall we look in the causal mechanism of the cells for evidences of the required differences? Practically put: Does increased complexity of structure in the phylogenetic series, as in individual development, involve more elements as well as increased extent of interrelationships between them? If, as seems apparent, each gene, representing a discrete unit of the determinative mechanism, corresponds to the incidence of some recognizable character in the completed organism, then it would logically follow that more characters mean more genes. This is probably a correct inference, in general terms, but at the same time we have to recognize the possibility of another alternative. If, as is certainly true, each form of organism is completely functional, then it possesses all the needed properties of an organism. "All that can happen toward progress is a refinement in the operation of these universal functions. Refinement, we find, commonly involves increased complexity in the mechanism which performs organic functions. Therefore apparently entirely new structures are only altered expressions of previously existing mechanisms. For instance, devices for producing motion in Metazoa consist, usually, of contractile elements attached to skeletal members. Motion results when muscles contract and force the skeletal structures to react against the opposing medium. Depending upon whether this medium is air, water, or a solid substratum, appropriate mechanisms develop. In

different cases if the same structural elements are involved we speak of "homologues"; if a similar device in form involves different structural elements we refer to them as "analogues." When only the contractile element is involved, greater diversity comes in, and this is further intensified when the usual paired members are replaced by a single median one. In view of the almost infinite range of motor mechanisms developed by organisms, it does not seem possible that they could result from the permutations of a common series of controls in the developing whole. At the same time when we recall that, simple or complex, they are each the result of an infinite number of repeated reactions between organisms and a given environmental requirement, the possibility of multiple modifications of a basic system must be considered.

Perhaps the problem might be stated specifically in this way: Since all organisms exhibit a common series of functions, and since functions are performed under the control of a recognizable series of agents within the chromosomes, there must exist a nuclear mechanism common to all organic types. Logically this would follow, and observation tells us that, at least in its major features, such a situation does exist, for cellular structure and behavior are essentially the same wherever found. Moreover, in the earlier and relatively simpler processes of development, strong likenesses prevail through all forms. But beyond this, what are we to expect? Does each advance in complexity, each new structural element mean additional gene controls or are they due to what might be called the better education of members of an existing system through new experiences? When it appeared that each character was due to the influence of a particular control there could be but one answer to this question, but now that it is known that each structure results from the operation of the entire integrated system, in which genes are merely differentiators, producing successively different results as development proceeds, then the picture is not so clear and simple. There must also be considered the facts that the cellular mechanism is always much the same, the number and size of the chromosomes is independent of phylogenetic stages, that different results follow upon changes of position of the genes in the series, and that probably the result of gene action varies with the time of its incidence. All these considerations emphasize the need for a most careful study of all elements of

the problem. Present knowledge would suggest, certainly, the existence of a basic and prevalent causal mechanism and at the same time the probability of the addition of new members in response to the demands of new conditions. Since, however, each gene modifies the action of the whole system, and therefore produces many effects, an equal number of additional elements would not seem to be required. The course of evolution indicates that any change, to become established, requires innumerable embodiments and great periods of time. Above all things it appears that any successful change must be completely conformable to conditions within the system and also to those in the environment. The participation of chance or accident is strictly excluded. It is true that biparental reproduction intrinsically implies variation, but this is always within definite limits and concerns the permutations of existing elements rather than the addition of new ones.

These considerations are of a very practical character when programs of study are concerned. It is a truism that we see what we are looking for, and this is particularly true of microscopical studies. Therefore, as students of cytogenetics we are much concerned to know whether we should seek additions to the chromosome mechanism or whether we should turn our attention toward the detection of modifications within a prevailing type. For many years my students and myself have concentrated our studies upon the conditions found in the germ cells of one family of insects—the *Acrididae*—seeking to establish the relationships existing between observable changes in the structure and behavior of chromosomes and the associated body characters. Many of these have been noted and found to be constant under given conditions. They involve varied features of chromosome structure, behavior, and relations, but represent, not additions of new things, but modifications in a persistent system. They involve such matters as differences in chromosome associations and in fiber attachment, variations in relative time of extension and concentration of individual chromosomes, forms and sizes of chromosomes, relations to chromomere vesicles etc.

This Orthopteran family is a distinctive group, with clear and sharply marked characters. Such variations of form as exist in subfamilies, genera, and species are due to modifications of proportions and relations of the members of the family com-

plex of characters. Logically, therefore, there would not be expected in the control mechanism of development any marked changes or any additions, and these are not found. The picture here is entirely consistent with our present assumptions in cytogenetics. It is a question, however, as to what might be found in comparing the conditions in this limited and well-defined animal group with others of very diverse nature. Without any guiding principle to indicate the best method of investigation, it has seemed wisest to learn as definitely and fully as possible what we can of the modifications shown in one organic group. After enough correlations between taxonomic characters and germ cell structure have been studied there will doubtless emerge the outlines of some principle underlying all the observed conditions, and eventually this will become generally applicable. However difficult such studies of broad comparative character may be, they are absolutely essential to an understanding of the true nature of living processes. No amount of pseudo-philosophical speculation, based upon hasty and imperfect studies, can take their place.

We are certain of the continuity of the germplasm, and of its general nature as the material record of racial experiences. It seems evident that the hope for an understanding of racial and individual development waits upon a fuller knowledge of the nature and behavior of the visible elements which embody the germplasm; and upon the inferences concerning the activities of the ultimate conceptual units revealed by genetical and embryological studies. Only long-continued, systematic, comparative cytological studies can provide the needed information. A continuation of the exceedingly fruitful coöperation between cytologists and geneticists, which has marked the years of the present century, will, in time, inform us of the intimate nature of the germplasm.

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## Hereditary Status of the Rhizopods

By

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NOTHING is known as to Mendelian heredity in the Rhizopods. There is no knowledge of inheritance and variation in sexual reproduction. Knowledge of genetics in the Rhizopods deals with the production of diverse types, and their inheritance, without the sorting over, exchange, and recombination of the hereditary materials that occur in sexual reproduction and that give rise to Mendelian inheritance. It deals with heredity and variation in reproduction from a single parent—in vegetative reproduction. It has brought to light remarkable relations between variations of the parent and variations of the offspring, in such uniparental reproduction; relations quite foreign to Mendelian heredity.

In Rhizopods multiplying vegetatively there exist in any species many different races, different biotypes, diverse in their inherited characteristics, retaining their diverse characteristics as they reproduce. Such genetically diverse biotypes are most evident and best known in the shelled Rhizopods: in *Diffugia*, *Arcella* and *Centropyxis*. The knowledge of these matters is based mainly on the work of Jennings (1916), Root (1918), Hegner (1919, 1920), Reynolds (1923, 1924) and Jollos (1924). I shall illustrate the phenomena from *Diffugia*, since I am best acquainted with that organism.

In *Diffugia corona* there exist many races or biotypes, which differ greatly in the characteristics of the shell; in its size, in the number of spines which it bears, in the form and length of the spines, and in the number of teeth surrounding the mouth. These characteristics are well seen in the plate of Leidy's Rhizopods that is devoted to this species (this plate reproduced on the screen—Slide 1); they are extensively illustrated in the figures given by Jennings 1916 (see figures 1, 2, 3 of Jennings

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1916, the last-named reproduced in the second slide). Figure 7 from the same publication shows different races, having diverse combinations of the characters. Each row in this figure is a series of generations beginning with the individual at the left. All the individuals thus derived from a single individual by vegetative reproduction constitute a clone. As the figures show, in vegetative reproduction each clone retains on the whole its distinctive characteristics, so that the clones remain diverse.

Yet among individuals of the same clone there occur slight differences in the characteristics: differences in size, in number of spines, in length of spines, rarely in the number of teeth surrounding the mouth. In vegetative reproduction, therefore, the different clones reproduce in their main features true to type, yet with minor diversities among the individuals of each clone.

These diversities within the single clone have become the object of investigation. Are such diversities, produced in vegetative reproduction, in any degree inherited? Is this variation which occurs at vegetative reproduction a genetic factor of importance, or is it a mere surface phenomenon, signifying nothing for hereditary differentiation?

To obtain the answer to these questions, the differing individuals of a single clone are allowed to reproduce separately, and their descendants are compared. When this is done with single individuals, even though they differ greatly, usually there is no indication of inheritance of the differences. If from a single clone of *Diffugia corona* we breed on the one hand from large individuals, on the other hand from small individuals, we find usually that both sets produce offspring of varying sizes, but that the average size is about the same in the descendants of both sets. The differences in size seem not to be inherited. Again, in a single clone we breed on the one hand from individuals with few spines, on the other hand from individuals with many spines. We find that both sets of parents produce offspring with varying numbers of spines, and that the average number of spines is about the same in the two sets. That is, the differences between the parents seem not inherited by the descendants.

This is the result that has been reached by extensive breeding work not only in the Rhizopods but in other Protozoa.

Diversities among individuals all derived by vegetative reproduction from a single parent appear when examined in the way described, not to be inherited. Vegetative reproduction, in the Rhizopods as in man, seems to result in the production of sets of identical twins, the number of individuals in the set rising in the case of Protozoa to thousands or millions, instead of being limited to 4 or 5, as in man.

But when breeding is continued for many generations, and includes large numbers of individuals of the same clone, it is found that the statement of the case just made is not complete. We find in such long-continued breeding some degree of inheritance of the differences. If in a clone of *Diffugia corona* we breed for many generations only from individuals with long spines, while at the same time we breed another group exclusively from individuals with short spines, we find after the lapse of numerous generations that the descendants of the long-spined parents have on the average longer spines than the descendants of the short-spined parents. We find in other words that the difference in length of spines is, in slight amount, in faint degree, inherited in vegetative reproduction. The same result is reached if we base selective breeding on other characteristics: on the size of the shell, on the number of spines, on the number of teeth surrounding the mouth. In this way, by selective breeding within the single clone there were obtained by Jennings 1916, five hereditarily diverse races or strains, shown in his figure 19 (here reproduced on the screen). These five differing races have been derived from a single individual through vegetative reproduction. They continue to be diverse when vegetative reproduction is continued; the differences are hereditary.

Similar extended work in selective breeding has been carried out on other Rhizopods, and with similar results. Hegner (1919), Reynolds (1923), and Jollos (1924) have thus worked upon *Arcella*, Root (1918) upon *Centropyxis*. In these, as in *Diffugia*, obvious diversities among individuals of a clone are usually not inherited, yet if selective breeding is continued for a series of generations, it is found that inherited diversities are produced. This occurs both with relation to differences in normal characteristics, and to abnormal diversities, to deformities in shape and the like.

To what extent or in what degree are the parental peculiari-

ties inherited? The answer to this may be illustrated from the results of selective breeding for different lengths of spine in a clone of *Diffugia corona*. Parents with long spines were bred for many generations,—the short-spined offspring not being allowed to breed further. In another set, parents with short spines were bred for many generations, the long-spined offspring in this second set not being allowed to breed further. The average length of the longest spine was measured for the offspring of parents that had diverse spine lengths, in a number of different grades. The accompanying Table 1 shows the relation of spine lengths in parents to spine lengths in offspring.

The table gives at the left the spine lengths of the different classes of parents. For the race as a whole the mean spine length is 12.54 units. The parents diverge from this mean length; some in the minus direction, having shorter spines than the mean; others diverge in the plus direction, having longer spines. The main feature shown in the table is that *the offspring diverge from the racial mean in the same direction as do the selected parents, but less than do the parents*. Parents that are below the average produce offspring that are below the average,

TABLE 1

INHERITANCE OF SPINE LENGTH WITHIN CLONE 326 OF *DIFFUGIA CORONA*.—MEAN SPINE LENGTH OF PARENTS HAVING GIVEN LENGTHS OF SPINES.—UNITS OF LENGTH ARE EACH  $4\frac{2}{3}$  MICRONS.

(From Jennings, *Genetics of the Protozoa*, 1929, p. 239)

Spine Length of Parents	Number	Offspring Mean Spine Length
4-6.....	21	10.38
7-9.....	162	11.01
10-12.....	481	11.85
13-15.....	367	12.90
16-18.....	129	14.39
19-21.....	26	14.34
22-24.....	15	16.34
25-31.....	18	17.06

Mean spine length for all—12.54

but not so much below as are the parents. Parents that are above the average produce offspring that are above the average, but not so much above the average as are the selected parents.

Thus the offspring inherit, on the average, a fraction of the parental divergence from the racial mean. From such a table



it is possible to compute what proportion of the parental peculiarities are on the average inherited by the offspring. The computation shows that with respect to spine length in this clone of *Diffugia* the offspring inherit on the average almost exactly one-third of the parents' divergence from the mean of the race or clone (the proportion which computation yields is 31.8 percent). Thus the average characteristics of the offspring always diverge from those of the selected parents in the direction of the clonal or racial mean. Further, it is also true that the average characteristics of the offspring diverge from the racial mean in the direction of the parental divergence. From the racial mean they diverge in this case by one-third of the parental divergence. From the parental peculiarities they diverge by two-thirds of the parental divergence.

Such a divergence of the offspring from the parental characteristics was called, in the pre-Mendelian studies of heredity by Galton, "regression toward mediocrity" or "regression toward the mean." Inheritance of parental characteristics within a clone, in vegetative reproduction, thus occurs with "regression toward the racial mean." The meaning of this will be clear from examples. In our table, parents with spines 4.5 units above the racial mean produce offspring that average but 1.9 units above the mean. The offspring have regressed 2.6 units toward the racial mean. Parents with spines 7.5 units below the racial mean yield offspring that average but 2.1 units below the racial mean, so that the offspring have regressed 5.4 units toward the mean.

Inheritance thus occurs as if there were two factors at work. On the one hand there is the peculiar constitution of the individual parent, which in some way tends to give the offspring a constitution like itself. On the other hand there is the constitution of the race as a whole, tending to make the offspring like the mean of the race. The resultant in the characteristics of the offspring is that they have but about one-third the parents' peculiarities, regressing two thirds toward the racial average.

These relations are similar to those which were discovered and emphasized by Galton in his pre-Mendelian studies of human inheritance. On them were based not only Galton's concept of inheritance with regression toward mediocrity, but also, in large measure, his "law of ancestral inheritance," which was

held to be fundamental in heredity. Galton was at work on biparental inheritance, in man. It is commonly held, I believe, that Galton's "inheritance with regression toward the mean" and the "law of ancestral inheritance," so far as valid, are indirect results of Mendelian inheritance in biparental reproduction,—taken in connection with non-heritable effects of environmental conditions. But here in the Rhizopods we find them in uniparental, non-Mendelian inheritance. Their nature and interpretation again become a problem.

This same type of inheritance—inheritance to a certain extent of parental peculiarities, but with partial regression to the racial type—occurs in the Rhizopods also with respect to other characteristics. In *Diffugia corona* this is true for size of the body; that is, for diameter of the shell. In a certain clone in which 2129 parents and offspring were measured (Jennings 1916, page 407), the offspring were found to inherit a little more than one-half (58.5 percent) of the parental peculiarities in this respect; they regressed toward the racial mean by a little less than one-half (41.5 percent). In this same clone the amount of the parental peculiarities inherited was for the number of spines present on the shell but about one-sixth, while the regression toward the racial mean was about five-sixths. Thus different kinds of characteristics differ in the degree of inheritance.

It is further notable that different clones, different biotypes, of the same species show different degrees of inheritance for the same class of characteristics. In some clones there is much variation and a high degree of inheritance of the diversities; in others little variation and little inheritance. This is illustrated in the following Table 2, showing the extent or degree of inheritance by the offspring of the parental peculiarities in shell size in different clones of *Diffugia corona* (from Jennings 1916).

Thus in some clones but about 27 per cent of the parental peculiarities was inherited; in other clones up to 58 per cent, or even 66 per cent.

Although numerical relations have not been worked out for other species it is clear that the same type of inheritance occurs in other Rhizopods. That is, in vegetative reproduction there is partial inheritance of parental peculiarities, but with partial regression to the racial type. This appears in the studies of

TABLE 2

PERCENTAGE OF THE PARENTAL DIVERGENCE FROM THE RACIAL MEAN IN DIAMETER OF THE SHELL THAT IS INHERITED BY THE IMMEDIATE PROGENY IN DIFFERENT CLONES OF *DIFFLUGIA CORONA*

(From Jennings, 1916)

Designation of Clone	Number of Individuals	Percentage of Parental Divergence Inherited	Percentage of Regression Toward Racial Mean
187 .....	226	34.2	65.8
303 .....	468	26.9	73.1
314 .....	960	26.9	73.1
317 .....	168	65.8	34.2
226 .....	2129	58.5	41.5

Reynolds (1923) and Jollos (1924) on the inheritance of abnormalities in *Arcella*. Certain relations shown in these studies may be examined from this point of view.

Several types of shell abnormalities were followed in the reproduction of *Arcella*; two of these types are shown in figures 29 and 30 of Jennings 1929 (reproduced on screen). Different degrees of abnormality occurred, so that a number of grades could be distinguished. One of the abnormalities was a bending or folding of the shell, giving rise to lateral lobes. Jollos in his work on *Arcella* distinguishes three grades, illustrated by the first three figures (on the screen). There exist of course many intermediate conditions, so that the classification into three grades is to some extent artificial.

Jollos practiced for many generations selection for higher grades of abnormality—breeding in all cases from the most abnormal individuals—those of the highest existing grade. He summarizes the progress of the experiment in the following table 3 which illustrates important features of inheritance (table 10, p. 264, Jennings 1929—Genetics of the Protozoa).

The table shows inheritance with regression toward the usual racial condition—which is here the normal form of the shell. Jollos calls this Grade O. The parental grade selected for breeding in each generation is shown by the point of the brace; thus in the first and second selections the parents were of grade 1, in the third to the sixth they were of grade 2, in the rest of the selections they were of grade 3.

The nature of the offspring resulting from each selection is shown in the columns numbered at the top. It will be ob-

TABLE 3

*ARCELLA POLYPORA*. CHANGE IN DEGREE OF ABNORMALITY OF THE DOUBLE-LOBED ANIMALS UNDER SELECTION FOR A HIGHER DEGREE OF ABNORMALITY; CULTURAL CONDITIONS NORMAL—THE GRADE 0 SIGNIFIES A FULLY NORMAL SHELL, GRADES 1 TO 3, SUCCESSIVE HIGHER DEGREES OF ABNORMALITY—AT EACH SELECTION THE MOST ABNORMAL INDIVIDUALS WERE EMPLOYED FOR FURTHER BREEDING. THE BODY OF THE TABLE SHOWS THE NUMBER OF INDIVIDUALS, OUT OF A SAMPLE OF 500, THAT BELONGED TO EACH GRADE AT EACH SELECTION

(From Jollos, 1924, page 334)

Selection	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grade																
0																
1	1	179	184	130	98	55	10	1	2							
2		321	310	328	326	259	203	111	84	4	3					
3			6	42	76	186	297	330	374	371	309	228	61	2		
								49	42	124	186	272	439	478	35	500

Mean Grade 1 0.64 0.64 0.82 0.95 1.26 1.59 1.83 1.91 2.24 2.36 2.54 2.88 2.95 2.93 3.

served that in all cases except the last one, the offspring are on the average lower in grade than the selected parents. That is, there is always (except in the one case) an admixture among the offspring of lower grade individuals. The mean grade of the individuals is given in the lower set of figures: it is in every case (except the one) lower than the grade of the selected parents. "Lower" here means nearer the condition normal for the race. That is, inheritance occurs with regression toward the racial norm.

But this table of Jollos shows also another relation of great importance: one that we have already seen in *Diffugia*. By continued selection of individuals of a certain type, in time a biotype is established of that kind. In inheritance there is not only a certain amount of regression toward the racial type. There is also inheritance of some portion of the parental peculiarities; inheritance of a part of the parental divergence from the racial type. Jollos in every generation selected for breeding the parents with the highest degree of abnormality. It must be remembered that the classification into three grades is rough and artificial; in fact the degree of abnormality of the selected parents increases steadily through the experiment. In consequence the grade of the offspring gradually rises also, though it is never so high as that of the selected parents. The lower row of figures shows this gradual rise in the grade of abnormality of the offspring. Finally, after the last selection,

the offspring were all sufficiently abnormal to be classified in grade 3, the highest grade distinguished. Beyond doubt the parents, though classified in grade 3 also, were more abnormal than the average of their grade 3 offspring.

It was by this same process of selecting parents showing characteristics of a certain type that there were produced the five diverse biotypes from a single clone of *Diffugia corona*, which were seen earlier. But in *Diffugia* there was selection not for one type only, as in Jollos' experiments with *Arcella*, but for several different types, so that five biotypes with different combinations of characters were obtained.

And this may serve to remind us of a very important feature of selective breeding in vegetative or uniparental reproduction. Such selection does not cause the production of any types or characteristics that would not be produced by unrestricted multiplication without selection. It merely causes the omission of some of the intermediate types that would have been produced. If vegetative reproduction occurred without restriction and without selection, all the types that are produced under selection would still be produced; also many others. Therefore, what the experiments show is that the single clone gradually splits up into many hereditarily diverse types, by the method I have described: by the occurrence of variation in vegetative reproduction, and by inheritance of a part of the diverse characteristics, along with partial regression to the racial type.

What is to be said as to the permanence of the diversities of biotype produced in this manner?

First, it must be observed that the biotypes found in nature, with which we begin our experimental breeding, are not permanent, are not unchanging. It is true that they retain their characteristics in the main for numerous generations; this is seen when we compare side by side two differing biotypes. But also they slowly differentiate into slightly diverse biotypes, in the way already described. Each of these new diverse biotypes likewise slowly differentiates into still different biotypes, just as did the parental races. There is no absolute permanence, no unchangeableness, anywhere. But there is a limited continuance of the different combinations of characters. Such a continuing combination is what we call a race or biotype. There are two tendencies at work, one conservative, shown in the partial regression toward the racial type, the

other toward novelty, shown in the partial inheritance of parental deviation from the norm.

By taking advantage of one or other of these tendencies, selection may isolate new combinations of characteristics, new races or biotypes. Or it may cut off the divergent types, causing the race to retain or to return to, its previous norm. This is what happened when abnormal stocks of *Arcella* were allowed to breed freely, without artificial selection, in the experiments of Jollos. He obtained by selection in the way described a population of five hundred very abnormal individuals of his grade 3. These were then allowed to breed freely, without external selection. They differentiated as always into more normal and less normal individuals. The abnormal individuals are under a disadvantage, for they do not retain easily the normal position with mouth against the substratum, cannot well creep about to obtain food, and they multiply more slowly than the normal individuals (Jollos, 1924, p. 338). In consequence of this, the proportion of abnormal individuals decreases; the population as a whole gradually approaches the normal condition. But after a year of this free breeding there continued to be a considerable proportion of abnormal individuals of various grades. If, however, the natural process is aided by artificial selection of the most nearly normal individuals for breeding, in the course of a year the entire population has become normal.

In the work on selection in *Diffugia corona*, on the other hand, no combination is abnormal and none of the new biotypes have obvious advantages over the others. Under these conditions there appears to be no ground for supposing that they would return to the original type if allowed to breed freely, without artificial selection. It appears that the process of racial differentiation would simply continue based on the three factors:—variation at uniparental reproduction, partial inheritance of the diversities, and partial regression toward the racial norm.

There remains an important question on which evidence exists. To what are due the variations that occur in vegetative reproduction and that are inherited in the way just described? These variations are the primary feature in the differentiation that occurs: how are they brought about?

There is evidence that in some cases these variations are pro-

duced, or at least influenced, by environmental conditions. Such a case is seen in the inherited abnormalities of the shell in *Arcella*, studied by Jollos (1924). These are influenced in their frequency and extent by environmental conditions. They become more frequent and more marked when the animals are cultivated in a small amount of fluid, not frequently changed, so that there is an accumulation of excretory products. But even under these conditions the abnormalities are produced only in clones which have a constitutional tendency to produce abnormalities, as shown by the fact that a few abnormal individuals occur in them even under the best of conditions. But the number and degree of the abnormalities are greatly increased by breeding in old culture fluid.

Now, what is of much interest, the fixity in inheritance of the abnormal characters depends on the length of time that the reproducing clones are kept in the conditions that favor the abnormalities. The longer they have remained in the old culture fluid, the longer the abnormalities are inherited under reverse selection.

This was shown by Jollos in the following way. By selection he had produced a set of individuals of which all were very abnormal: all showed grade 3 of the abnormality. As we saw earlier, such very abnormal clones can in the course of time be brought back to complete normality by selecting for breeding only the most nearly normal individuals; but this required some months of selective breeding. Now different portions of such a completely abnormal clone were subjected for different periods to the old culture fluid that favors the abnormality; then each was brought back to normality by a long process of selective breeding. The point of interest is that the longer they had been subjected to the conditions favoring the abnormality, the longer was the time required to bring them back to normality through selective breeding. Those that had been four months in the old culture fluid required two months of selective breeding to bring them back to normality. Those that had been seven and one-half months in the old fluid required three months; those that had been twelve months in the old fluid required four months to bring them back to complete normality. The effect of the old fluid in producing abnormality is according to the observations of Jollos inherited for many generations after the old fluid has ceased to act.

This is one of the examples of what Jollos calls "Dauermodifikationen": environmental modifications that are inherited for many generations, but finally disappear. More numerous and more satisfactory examples of these "long-lasting modifications," inherited but finally disappearing, are to be found in other divisions of the Protozoa: particularly in Flagellata and Ciliata.

Reynolds (1924) made by an ingenious method a study of the gradual coming on of hereditary differences through the action of different environments. In *Arcella*, when a piece of a pseudopod is cut off from an individual, it will after being isolated a short time again coalesce with that same individual when the two come in contact. But if the isolated pseudopod comes in contact with another individual, in many cases the two will not unite. Often indeed there is what Reynolds calls a "shattering reaction," the isolated pseudopod and that piece of the other individual with which it comes in contact break into small bead-like droplets.

If individuals are closely related, and have lived under the same conditions, an isolated piece of one readily coalesces with the other. But if two such closely related individuals are kept for some time under different conditions, after a certain period parts of the two will no longer coalesce. The two have become in some way slightly diverse, so that the protoplasm of the two will no longer unite. Two sister individuals, with their offspring kept one in hay infusion, the other in sugar solution, remain in condition to unite for seven or eight days. But after such a period they and their descendants will no longer unite with the individuals that have been living under the other conditions.

If now these individuals that have thus become negative to each other are placed together under the same conditions, they remain negative for 6 or 7 days, 6 or 7 generations. The diversities produced by different environments are inherited. But after 6 or 7 days together the common environment has caused them to become alike, so that pieces of the two unite when they come in contact.

Also, unrelated individuals, which are originally negative to each other, become positive, become sufficiently alike to unite when they come in contact, if they are left for about nine generations in the same drop of culture medium.

Thus in Reynolds' experiments we see the delicate begin-



conditions. The experiments could be carried farther with profit, to determine whether longer-lasting hereditary differences could thus be produced.

There remains the question: Is it the chromosomal materials, is it the genes, that become altered when hereditary diversities are produced within a single clone, through the action of the environment or otherwise? Jollos is of the opinion that it is *not* the chromosomal materials that are altered, since mutations produced by the alteration of chromosomal materials are usually very constant in the characteristics that they present, while the hereditary changes occurring in the Rhizopods are, as we have seen, notably inconstant. But on this matter there is no evidence derived from the Rhizopods. In any case, it can be settled only by working out the system of inheritance in reproduction from two parents. That will be more readily done in other divisions of the Protozoa.

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# Nuclear Behavior and Reproduction in Ciliated Protozoa

*By*

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REPRODUCTION and its associated nuclear phenomena in the ciliated protozoa are perhaps more atypical and puzzling than in any other one large group of animals. Two conditions contribute to our peculiar interest in the reproductive behavior of ciliates. First of all, they are complete and fairly highly specialized animals, and at the same time single cells: the parts of the cell, its physiological activities (youth, maturity, old age), and the external environment react on each other directly. In the second place, the majority of the ciliates possess a nuclear apparatus in which the chromatin is segregated into two distinct and different bodies: the macronucleus and the micronucleus. The former is regarded as vegetative, somatic, in function; while the latter is reproductive, germinal, in function. Genetically, the micronucleus produces the macronucleus: the latter has specialized and differentiated from the primitive embryological character of its parent.

Many ciliates, as well as Metazoa, begin their life cycle as a primordial cell which is the stage of minimal protoplasmic differentiation. From this primordial stage come to be differentiated epigenetically diverse structures with specific functions, derived for the Metazoa during many mitotic divisions but for the ciliates during one mitotic division and rederived, at least partially, during each succeeding division. Thus, ciliates retain the capacity to reorganize, that is, to dedifferentiate and redifferentiate, which they tend to do during fission, conjugation, gamete formation, autogamy, endomixis, encystment, and regeneration; and are accordingly potentially immortal. Metazoan ontogeny, however, proceeds toward a fixed and irrevers-

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ible state of differentiation which eventuates in disintegration and death.

Certainly the most common method of reproduction in ciliates is binary cross-division. This often involves a resorption and reformation of motile organelles such as cirri, membranelles, and even body cilia, as well as the renewal of certain old metaplastids, such as pharyngeal baskets (*Chilodonella*) and supporting structures (*Trichodina*). Nuclear changes during division are quite varied, each species having its own peculiarities of macronuclear condensation and reformation. Information is accumulating to the effect that the macronuclear substance often reorganizes profoundly at the time of its division. In some cases the macronuclear components undergo a phase reversal, that is, the chromatin granules which in the resting nucleus are dispersed in the non-staining fluid become the dispersion medium during division. In a number of hypotrichs there occur, prior to and during division, one or two characteristic zones which have been called by various names, such as nuclear clefts, reconstruction bands, reorganization bands, etc. In *Euplotes patella* (Turner '30) and in *Diophrys appendiculata*, *Stylonychia pustulata* and *Aspidisca* (Summers '35), the history of these bands during division was followed. Summers suggested that "the reorganization bands are local regions of karyolysis and resynthesis of macronuclear materials with the possibility of an elimination of physically, or possibly chemically, modified non-staining substances in the cytoplasm." The discarding of a certain portion of the macronuclear material during division has been observed in a number of ciliates, largely by Kidder and his co-workers. They believe that the process is one of elimination of waste substances accumulated during prolonged cell division, since chromatin extrusion does not take place during a few divisions subsequent to reorganization after conjugation in *Conchophthirius mytili*, and since in *Colpidium* and *Glaucoma* the chromatin elimination appears to be correlated with high division rate and infrequency of conjugation. It is possible that every fission is to some degree a rejuvenating process.

The micronucleus in ciliates (both in fission and in all other activities), in contrast, always behaves mitotically. However, since the nuclear membrane does not break down an intranuclear spindle is formed. Chromosome numbers vary from four to several hundred. According to the majority of reports on

ciliate mitoses, the chromosomes split longitudinally yet there are observations which suggest a transverse division. In these cases it has been proposed that each chromosome contains but one gene. In *Zelleriella intermedia*, Chen ('36) was able to determine the individuality of each of the twelve pairs of chromosomes and to see their longitudinal splitting. On certain of them a nucleolus is constantly present.

The Protociliata represent, probably, the most primitive Ciliophora and as such are of some interest in this discussion. They are inhabitants of the intestine of Anura, primarily. There is no mouth. The number of nuclei varies from two to several hundred, all of which are of one type. They are practically the only ciliates which do not have dimorphic nuclei. Asexual reproduction is by binary fission. The method of sexual reproduction by copulation of gametes and not by conjugation also distinguishes the protociliates from other ciliates. Individuals begin to divide rapidly with decreasing size until there result minute forms with one, two, or more nuclei, according to the species. These encyst and pass out with the feces. According to Metcalf, in the later mitoses preceding encystment the number of chromosomes is reduced. Tadpoles ingest the cysts which open in the rectum, giving rise to the same type that had previously encysted. These now multiply, ultimately forming uninucleate gametes of two sizes. One of each copulates and fuses completely with the other. The zygote either encysts again and passes out to infect other tadpoles or develops at once into the adult. Metcalf has pointed out the need for further investigation of the nuclear details preceding gamete formation and copulation. Many students regard the condition of uninucleate gametes which copulate as being the most primitive type of syngamy from which conjugation (temporary union of gamonts) has been derived.

Possibly the next step in the evolution of reproductive practice in the ciliates might be illustrated by a life cycle such as that of *Ichthyophthirius multifiliis*. In this holotrich the life cycle is in two distinct parts. The young, undifferentiated ciliate penetrates the skin of a fresh water fish, develops a functional mouth, feeds, and grows (in from four to eight days). The full-grown mature ciliate reaches a diameter of 100-1000 $\mu$ , then drops off and encysts. It undergoes a series of rapid divisions until each daughter is less than 40 $\mu$  in diameter and 100-1000

individuals are produced in each cyst. Nuclear reorganization then takes place, probably by a process of autogamy (Haas '33 and MacLennan '35), and the young reorganized ciliate, usually called a ciliospore, escapes from the cyst and enters the fish. Reproduction takes place normally only within the cyst. It is not clear whether the reported autogamous process within the cyst involves fusion of discrete cells of related origin or fusion of the micronuclei (which are present during all stages of the life cycle) belonging to a single cell. Details of possible chromosome reduction and restoration have not been given. This type of life cycle is similar, at least superficially, to that of many gregarines. In a careful study of the history of cytoplasmic bodies, MacLennan ('35) reports that large basophilic granules of protein are formed within the macronucleus and are extruded during the feeding cycle and early encystment. Also at various times during encystment, large ball-like masses of macronuclear chromatin are extruded.

*Glaucoma (Dallasia) frontata*, a free-living holotrich, illustrates a most unusual sexual behavior—probably a further evolutionary step. Here there are two types of fertilization: one by the fusion of autogamous gametes (copulation series), the other by typical conjugation. Calkins' and Bowling's general interpretation of this life history is interesting. They believe that phenomena analogous to the double series of *Glaucoma* occur in the life cycles of practically all ciliates. In the ciliates as a group, these are the periods of encystment, endomixis, conjugation and other reconstructions during each of which more or less complete reorganization takes place. The copulation series of *Glaucoma* is the history of an actual gamete brood ending in paedogamous copulation. The conjugation process is interpreted as a rather divergent manifestation of typical fertilization processes as carried out by other protozoa, and a reminiscence of an earlier gamete brood formation. Evidence for this conclusion comes from a number of considerations: the supernumerary potential pronuclei in many cases, spermatozoa-like pronuclei in certain species, etc. If an ancestral gamete brood is equally true of the conjugation series and of the copulation series, then both series are phenomena having the same evolutionary significance. Furthermore, they reason, the copulation phase of *Glaucoma* is an aggregate of phenomena that has the same significance for *Glaucoma* that endomixis without

encystment has for *Paramecium* or that endomixis with encystment has for other ciliates.

Further theoretical evidence relating conjugation to an earlier gamete-copulation mode of reproduction is furnished by diverse examples, such as the holotrich *Cryptochilum* (Russo '26), by certain oligotrichs (Dogiel '25) and by the peritrich *Vorticella* (Maupas '89). In these forms special divisions of the body precede actual conjugation so that the conjugants are smaller and in some cases otherwise different from the non-conjugating population. As a matter of fact, it is a regular occurrence for conjugants to be smaller than non-conjugants, e.g. *Nyctotherus* (Wichterman '36). Particularly interesting conditions are found in the commensalistic oligotrichs (Dogiel '25). Here, in many cases, an unequal preconjugant fission, the last before conjugation, produces one macroconjugant and one microconjugant which differ also in morphological details. In this preconjugant division, comma-shaped micronuclei have been described, indicating that the meiotic process has been initiated before the prospective conjugants have paired. This behavior is suggestive of autogamy. What the history of such preconjugants would be if conjugation were prevented is not known. "Over-ripe" conjugants were described. During pairing the number of micronuclear divisions characteristic of other ciliates is abbreviated. Also, the wandering pronucleus has a sperm-like shape. It develops a tail and makes its way through the membrane of the peristomal region of the mother-cell into the external chamber formed by the head-on fusion of the two conjugants (fig. 1). From this chamber it enters the other conjugant by way of the mouth and pharynx and ultimately meets and fuses with the stationary nucleus of this conjugant. Interchange and fertilization are reciprocal in both members. It is logical to regard these gametic nuclei as rudimentary gametes (especially the migratory gametes) which are acquiring something of the cytosomal specialization characteristic of metazoan germ cells.

At the end of such a series of conjugations between unequal members should be mentioned conditions in *Metopus* and *Vorticella*. In the former, a free-living heterotrich, the two conjugants are similar at the beginning of the process, but soon show a difference in behavior (Noland '27). The early micronuclear divisions are equivalent in each, and typical, but at the time the pronuclei are formed the cytoplasm and both pronuclei

of one conjugant pass into the other, leaving the degenerating macronucleus and a small amount of cytoplasm behind in the shrunken pellicle of the smaller conjugant (fig. 2). The latter separates and dies. In the larger ex-conjugant, two pronuclei fuse; the other two degenerate and disappear. The evidence was not clear as to which two of the four pronuclei fuse to form the synkaryon. In any case, only one of the conjugants is genetically persistent as a separate organism and the process is either cross-fertilization or autogamy, depending on the ancestry of the synkaryon.

In the vorticellids, certain of the ordinary attached individuals serve as macroconjugants, while others by two or three rapidly succeeding fissions give rise to small free-swimming microconjugants. One (frequently two) of these attaches itself to the side of a stalked individual (fig. 3). In the latter, the usual three micronuclear divisions give rise to the pronuclei. In the microconjugant an extra preliminary division is interpolated. The wandering nucleus of the smaller conjugant unites with the stationary nucleus of the larger conjugant; the other two pronuclei degenerate. The small remnant of the microconjugant left after conjugation drops off and dies. This type of life cycle has been thought to be an adaptation to the sessile mode of life, but this can hardly be the entire explanation in view of a somewhat similar life history in the free-living *Metopus* and the occurrence of normal conjugation in attached forms like the Suctoria.

In by far the majority of ciliates, conjugation occurs in a manner illustrated typically, I think, by *Paramecium*. This process is peculiar to the Euciliata and Suctoria, the morphological result of which is a replacement of the nuclear apparatus of each member by products of the activity of the micronuclei. Maupas ('89), the first to make a comparative study of different ciliates during conjugation, described eight successive phases of the process which are still applicable to practically all ciliates. The first four of these phases have to do with the phenomena of maturation, the last four with the process of reorganization of the individual. In many ciliates in which the history of maturation has been followed there is very little to distinguish the first maturation mitosis from the usual vegetative divisions, beyond a slight swelling. But in the majority of ciliates this first maturation division is markedly different from somatic



mitoses. In most species of *Paramecium*, both those with a massive and those with a vesicular micronucleus, a typical prophase stage occurs in the form of a long crescent which may extend half the length of the cell. Another distinctive type of prophase is the "candelabra" or "parachute" nucleus exemplified by *Euplotes* (Turner '30).

In many ciliates, a reduced chromosome number is reported to occur in the metaphase of the second division, e.g. *Chilodonella* (MacDougall '35) and *Uroleptus* (Calkins '19). In all cases the second meiotic division appears to be unaccompanied by any of the preliminary activities which characterize the first division. In most instances the nuclei do not return to a resting condition between the two divisions. Accumulating evidence on meiotic phenomena in the ciliates points to the conclusion that their history in the main is similar to the history of meiosis in Metazoa. Chromosome numbers are observed to be reduced to one-half during one or the other of the two divisions. As in the Metazoa, two divisions seem to be required to accomplish reduction. Accordingly, there appears to be no basic reason in the protozoa why the appearance of a reduced chromosome number should be restricted to either the first or the second division.

A third division of the nuclei subsequent to reduction is characteristic of all ciliates in which fertilization has been carefully studied. It appears to be an equational division, as in *Oxytricha fallax* (Gregory '23). This final division which gives rise to the pronuclei is difficult to interpret. It finds no counterpart in the Metazoa unless one assumes that the gamete-differentiating processes in the Metazoa replace the extra nuclear division of the ciliates. There is no rigid uniformity in regard to the number of nuclei which engage in the third division. In *Paramecium*, as a rule, only one nucleus goes forward at this time. However, in *P. aurelia* the writer ('36) found all numbers up to five and in *P. caudatum* (the writer '40 and Wichterman '40) found varying numbers up to all four of the products of the second division completing the third division. It has been thought generally that only one of the dividing nuclei provides the two functional pronuclei. This is a difficult matter to settle cytologically, in all cases.













The interchange and union of pronuclei have been considered to be the significant features of the process, resulting

in reciprocal fertilization. Cytoplasmic continuity between the two conjugants is established in many cases by the dissolution of the cell membranes in the region of contact. Often, connecting spindles between the two sets of pronuclei stretch over the protoplasmic bridge so that there can be no doubt as to the actual interchange. Sometimes, moreover, even the macronuclei extend across the bridge from each member to the other and parts of them are exchanged, as in *Anoplophrya* (Collin '09) and *Chilodonella* (MacDougall '35). Macronuclear passage appears to follow micronuclear transfer. In *Paramecium*, conditions seem to be different. The period of cytoplasmic continuity is certainly one of short duration. Paroral cones are formed early in the conjugation process and these may or may not break down to allow passage of the pronuclei. Wichterman ('39) studied living pairs of *P. caudatum* and by direct observation found that transfer of pronuclei did not occur; instead, the gametic nuclei of a given joined individual fused and formed a synkaryon in the same individual. He has proposed the term "cytogamy" for this phenomenon to distinguish it from autogamy (of the type which occurs in single unpaired individuals) and from conjugation of the type which involves a nuclear transfer in joined pairs. His observations have led Wichterman "to question the work of others where a transfer of pronuclei is presumed to occur in 'conjugation' of *P. caudatum*, especially when the contiguous membranes of the two joined individuals are not cytolized." Perhaps cytogamy and classical conjugation are racial or culturally induced characters, both of which may be found within the same species. My experience with fixed material has convinced me of the reality of both processes; in most of the cultures of *P. caudatum* I have studied there was not clear evidence of gametic transfer; in others there was strong cytological evidence of cross-fertilization. *P. trichium*, also, seems to be variable in this respect; some stocks exhibit macronuclear interchange in late stages of conjugation (fig. 4), while others do not. Moreover, micronuclear migration in this species sometimes occurs at an improper time in the conjugation process. With respect to the question of interchange of nuclei, the work of Chen ('40) on *P. bursaria* is pertinent. In the majority of cases of the conjugation of a micronucleate and an amiconucleate individual there was transfer from the former to the latter of a gametic nucleus, a process resulting in a re-

duced chromosome number in each conjugant. In a small number of cases no such transfer took place, the two pronuclei remained in the same conjugant and fused to form a synkaryon (autogamy or cytogamy). The pairing of two individuals with distinguishing types of micronuclei, in other experiments by Chen on *P. bursaria*, demonstrate conclusively the exchange of pronuclei in these matings. His findings on triple conjugations are interesting. Two members of the triplet exchange pronuclei while the third undergoes autogamy.

The synkaryon is the basis of the new nuclear apparatus. By appropriate divisions the nuclear condition typical of the species is restored. Thus the synkaryon may divide only once, and the two nuclei may become the single macronucleus and micronucleus of the adult so that the normal condition is reached without further divisions, e.g. *Metopus*. Usually the reconstruction is more complicated.

A reproductive process in *single* ciliates, somewhat paralleling the phenomena of conjugation, was recorded as endomixis by Woodruff and Erdmann ('14). This was described as "a complete periodic nuclear reorganization without cell fusion in a pedigreed race of *Paramecium*." At intervals of approximately thirty days, these investigators found that the old macronucleus of *P. aurelia* gives rise to fragments which are absorbed in the cytoplasm. Each of the two micronuclei divides twice, forming eight products. After degeneration of six or seven of these, the remaining ones reconstitute new micronuclei and macronuclei that are distributed by means of fission until the normal condition is attained. Chromosome reduction was believed to be absent and all cases of endomixis were distinguished from conjugation and autogamy by the absence of the formation of a synkaryon. The occurrence of endomixis both during free life and during encystment has since been described in a great many ciliates. Most of the accounts agree in their failure to find maturation and fertilization phenomena, thus conforming to the essential criteria of endomixis. The author has been studying *P. aurelia* for a number of years, cytologically, and in the races examined has not been able to identify the process of endomixis. Recently ('36) I found, instead, the process of autogamy (text figure I). This takes place in single animals and seems to be the exact morphological equivalent of conjugation. Maturation and fertilization occur as in conjugation; nuclear

- A**  Typical animal with two micronuclei and a macronucleus.
- B**  "Crescent" micronuclei. This is a characteristic prophase of the first prezygotic division.
- C**  Second prezygotic division. Four micronuclei; two of them represented as lying in the concavity of the cup-shaped macronucleus.
- D**  Eight micronuclear products of the second division. Macronucleus preparing for skein formation.
- E**  Variable numbers of nuclei continue to divide a third time. In the case illustrated, two are functional; one lies in a bulge near the mouth ("paroral cone").
- F**  Potential gamete nuclei arise after the third division. Degenerating nuclei from the second and third divisions may be present.
- G**  Two gamete nuclei fuse in the paroral cone. Synkaryon formation.
- H**  First division of the synkaryon.
- I**  Second division of the synkaryon. Macronucleus fragmenting.
- J**  Four products of the second division. Two transform into the new macronuclei and two remain micronuclei.
- K**  Macronuclear anlagen well developed. The first cell division under way. The micronuclei are dividing.
- L**  Macronuclear anlagen segregated to the two daughter cells. Old macronuclear fragments disintegrating.

TEXT FIGURE I.—Diagram of the nuclear changes during autogamy in *Paramecium aurelia*.

reconstruction is accomplished in the same manner, and autogamy would appear to be a zygotic type of parthenogenesis. How general is the occurrence of autogamy among the ciliates remains for future work to determine, but it seems to be very common in many races of *P. aurelia* though rare or absent in all the other species of *Paramecium* which I have examined. Probably the alternative of conjugation or autogamy is associated in some way with the mating reaction. Another question is the evolutionary relationship between autogamy and conjugation. Has conjugation in *Paramecium* arisen from autogamy or vice versa? It is hardly conceivable that these two processes could have evolved independently.

An interesting recent development in the field of ciliate cytology has been the discovery of heteroploidy within the species. The first demonstration of this phenomenon was given by MacDougall ('25, '29) in *Chilodonella uncinata*. In a series of polyploid forms she described diploids with a chromosome number of four, triploids with six, and tetraploids with eight chromosomes. Chen ('40) has reported polyploidy in *Paramecium bursaria* and *P. caudatum*, and Diller ('40) heteroploidy in *P. caudatum* (fig. 5), *P. trichium* (fig. 6), *P. multimicronucleatum* (fig. 7) and *P. aurelia* (fig. 8). In *P. bursaria*, Chen found a great range in polyploidy from a possible diploid number of 80 up to several hundred. He gives as the most plausible explanation of the origin of polyploidy in *Paramecium* the fusion of more than two pronuclei. Hamburger ('04) showed a figure of a pair of conjugants of *P. bursaria*, one member of which had a synkaryon composed of three gametic nuclei; the other conjugant had a single gametic nucleus (fig. 9). Chen saw a number of cases of the presence or fusion of three or four pronuclei in each conjugant. Hypoploidy, on the other hand, may have arisen as the result of anomalous maturation behavior or the failure of synkaryon formation to occur (gametic parthenogenesis), or a combination of these two causes. I have some inconclusive evidence in *P. trichium* and *P. multimicronucleatum* to suggest that gametic parthenogenesis regularly occurs in hyperploid stocks. Some races of *P. aurelia* are characterized by a normal sized micronucleus and a small one. It is possible that the larger one has had a synkaryon ancestry while the small one may have developed from a parthenogenetic pronucleus.

Apparently, the nuclear condition of preconjugants has very little, if any, direct effect on the mating response. Müller ('32) has even reported the conjugation of *P. caudatum* with *P. multimicronucleatum*. Successive re-conjugations involving young undivided ex-conjugants are not uncommon. Most of the accounts in the literature on amiconucleate races have noted the absence of conjugation in them. However, in one amiconucleate race of *P. multimicronucleatum*, the writer found and studied matings in which the macronucleus behaved rather typically. This was followed by one hundred per cent mortality. Nor does the condition of heteroploidy seem to be causally related to the mating reaction. In a study of five races of one mating type of *P. bursaria*, Chen ('40) found that there appears to be just as much variation in chromosome number among races within the same mating type as between different mating types. He concludes that it is possible that mating type is inherited irrespective of chromosome numbers. Tartar and Chen ('40), in merotomy experiments with *P. bursaria*, found that enucleate

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1. *Opisthotrichum janus*. In the posterior end of each is the macronucleus and a degenerating micronucleus. Toward the anterior end the pronuclei are being formed. This last pregamic spindle is slightly more advanced in the macroconjugant than in the microconjugant. After Dogiel.

2. *Metopus sigmoides*. Pair after passage of pronuclei from the smaller to the larger conjugant. Two of the potential micronuclei are degenerating. After Noland.

3. *Carchesium polypinum*. Two microconjugants attached to a macroconjugant. One first maturation spindle in the macroconjugant. Two spindles in each of the microconjugants; they have completed their preliminary division. After Maupas.

4. *Paramecium trichium*. Interchange of macronuclear strands. A synkaryon and a degenerating micronucleus in each conjugant. Original.

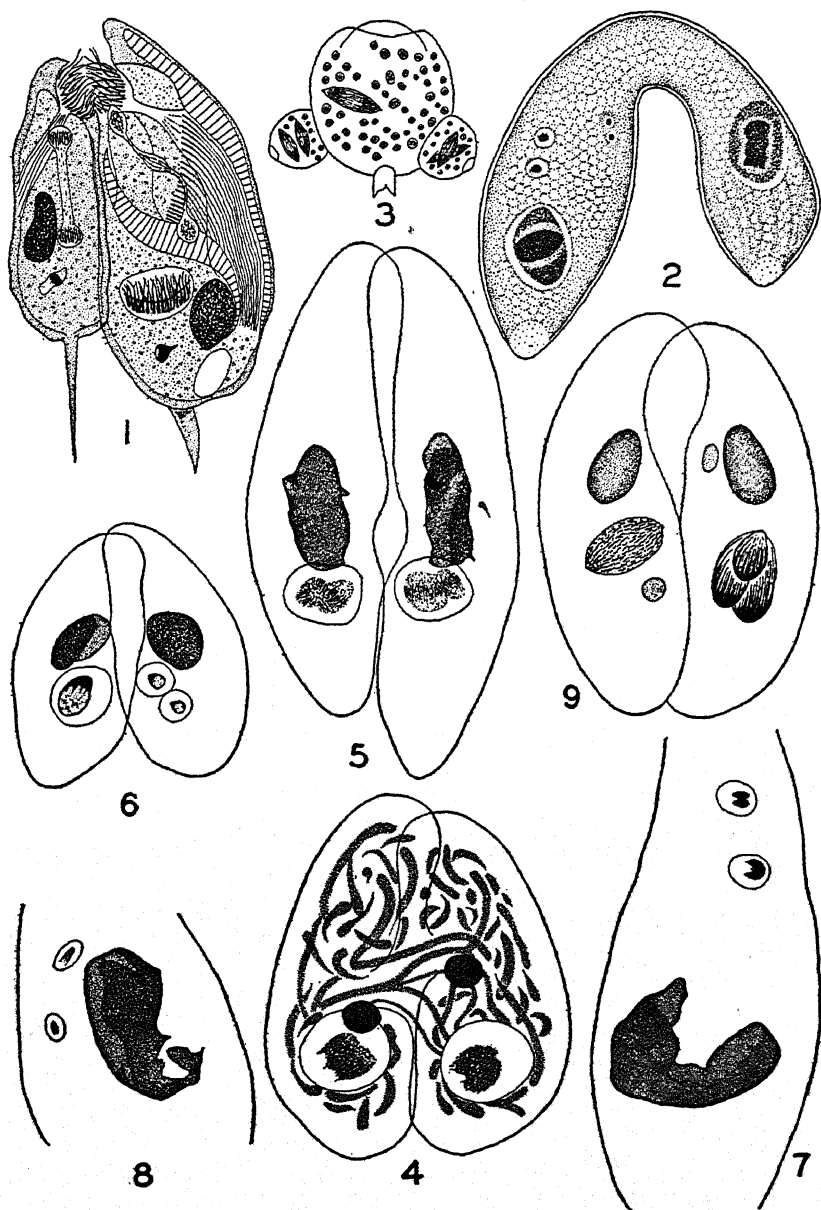
5. *Paramecium caudatum*. First maturation metaphase. Note the large, probably hyperploid, micronucleus with at least two groups of chromosomes in each. After Diller.

6. *Paramecium trichium*. First maturation prophase. Probably hyperploidy in left conjugant; hypoploidy in the two small micronuclei of right conjugant. After Diller.

7. *Paramecium multimicronucleatum*. First maturation prophase in conjugation (the other conjugant not represented). Note the double chromatin masses. Probably hyperploidy. After Diller.

8. *Paramecium aurelia*. Vegetative fission. Part of the anterior end showing prophase micronuclei. Note the differences in size and chromatin content of the two nuclei: probably hyperploid and diploid conditions. After Diller.

9. *Paramecium bursaria*. In the right conjugant, fusion of stationary and two migratory pronuclei. In the left conjugant, division of the single stationary nucleus. After Hamburger.



fragments of either mating type gave the normal mating reaction with whole animals of the other mating type.

Thus, though there occurs in the ciliates considerable variation in nuclear processes, these manifestations may conceivably be regarded as phases in the evolution of sexuality wherein we might trace a series beginning with dimorphic gamete formation in the protociliates, through autogamy (*Ichthyophthirius* and *Glaucoma*); then conjugation—in which both gamonts are hermaphroditic and the gametes (pronuclei) practically identical; until we culminate in the Ophryoscolecidae and Vorticellidae where the gamonts, and the gametes themselves, show sexual specialization approaching that of the Metazoa. Underlying all the apparent diversity and variability of nuclear phenomena one comes to discern a fundamental similarity whose expressions are differences in degree and not in kind.

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## Inheritance in Ciliated Protozoa\*

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HEREDITY in ciliated Protozoa is too complex to be portrayed fully or adequately in the time at my disposal. I have therefore selected for discussion what appears to me to be the most important and interesting problem of ciliate genetics: the problem of the basis of the observed hereditary diversities. In genetics of higher organisms we have learned to interpret all hereditary diversities as due to differences in the number, arrangement or kinds of genes present, except for a few instances of diversities due to differences in plastids. In the Protozoa, on the other hand, there are many observations difficult to reconcile with genic or plastid interpretations; but the great weight of evidence from higher organisms and the impracticability of carrying out the necessary genetic analysis long prevented these observations from leading to any solidly founded alternative interpretation. In recent years, however, genetic analysis has been facilitated and this has resulted in a rapidly growing body of knowledge that may lead to radically new genetic principles. I shall attempt to portray current and earlier work primarily in their relation to this.

The problem of the basis of hereditary diversities in ciliates may be studied in two kinds of material: during purely vegetative reproduction, when the genic constitution should remain constant; and at fertilization and nuclear reorganizations, when new genic combinations may be formed. In vegetative reproduction, it has been known ever since the pioneer studies of Jennings (8) that hereditary characters ordinarily remain constant. At first, all the products of uniparental reproduction were believed to constitute a genetically uniform clone; but later studies (2, 7, 12, 13, 15, 20, 21, 24, 25, 27, 28, 30) showed that hereditary characters may change at nuclear reorganizations involving only

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one parent; and some (2, 13, 15, 25, 27) showed that the individuals arising at the first few fissions after both uniparental and biparental reorganizations may differ in their hereditary characters. As these reorganizations may involve fertilization (6, 28) and as the immediately following fissions are not strictly vegetative (for diverse macronuclei are segregated, not divided), the clone, or group of vegetatively produced individuals, should not include them. These discoveries of hereditary diversities during uniparental reproduction thus served to call attention to the fact that we were including too much in the clone, but gave no evidence for the origin of hereditary diversities within the clone, properly conceived.

Many recorded observations do purport to show however that hereditary diversities may arise within such a clone. Their occurrence is almost as generally accepted as the principle of clonal uniformity itself. Yet I believe, as I shall attempt to show, that most of the evidence for it is not critical. In much of this work the possibility that the observed diversities arose at endomixis or autogamy, and so not strictly within a clone, has not been satisfactorily excluded. This objection holds for practically all the observations on *Paramecium* except those to be mentioned later.

In most of the remaining studies, the diversities observed are not clearly hereditary.\* This criticism may be applied to the genetic interpretation of the well-known "life-cycle" of ciliates. Each stage in the cycle—immaturity, maturity, senescence—is marked by a number of characters persisting through many cell divisions, and so superficially appearing hereditary. Comparable changes take place during development of a multicellular organism and, while their nature is but ill understood, presumably cytoplasmic differentiations arise as a result of progressive changes in nucleo-cytoplasmic interaction. Similar processes may underlie the life cycle changes in ciliates. They may also be at the bottom of the intraclonal diversities shown

\* Any definition of heredity is arbitrary and objectionable. To avoid misunderstanding, however, it is desirable to state explicitly how the term is here employed. In this paper determination by self-multiplying materials (either nuclear or non-nuclear) is considered to be the essential feature of hereditary characters; characters determined by non-multiplying materials passively distributed from cell to cell are considered non-hereditary. To demonstrate that a character is hereditary it is therefore held necessary to exclude the latter possibility.

in the so-called "cytoplasmic lag" effect (5, 16, 29). When hereditary characters change as a result of conjugation, autogamy or endomixis, the first individuals produced in the new clone may have the same characters as did the ancestor prior to reorganization. The new character first appears suddenly or gradually in individuals produced days or even weeks later. As not all lines of descent change simultaneously, diverse lines of descent may temporarily exist within the same clone. Until the possibility of progressive cytoplasmic changes under nuclear action is excluded, these cytoplasmic lag changes, like the life cycle changes, can hardly be considered critical evidence of intra-clonal hereditary diversity.

Changes indistinguishable from life cycle changes are producible (1) by inadequate cultural conditions in species that manifest no such changes under favorable conditions. Other environmental actions are known (13), resulting in acclimatization or increased resistance to various chemical and physical agents. Such changes are often gradually accumulated by prolonged environmental action. Though they commonly disappear at once, after removal from the inducing conditions, their loss also is sometimes gradual. As these gradual changes extend over many fissions, they have been considered by some to be hereditary. But this need not be if the environment produces effects on the cells more rapidly than they are being diluted by divisions. Though we must thus reject as uncritical all the types of evidence so far mentioned, there remain several studies in which the observed intracolon diversities are probably or certainly hereditary.

The earliest apparently unobjectionable example of intracolon hereditary diversity in ciliates is Middleton's (19) study of the effects of selection in *Stylonychia*: lines differing in fission rate were isolated within a clone by means of repeated selections. As endomixis and autogamy are unknown in *Stylonychia*, the observed diversities appear to be intracolon as well as hereditary.

Recently Kimball (17) brought to light another case of great interest. He observed that the mating types I and II in *Paramecium aurelia*, ordinarily strictly inherited within a clone, behave very differently in certain rare clones. Such a clone includes some individuals of type I, others of type II. Ordinarily any individual, of either type, will give rise by fissions to

progeny of both types. Though this looks as if the mating type is not an hereditary character in these clones, two further observations suggest otherwise. In the first place, there is some, although not perfect, correlation between the mating types of parent and offspring; usually the vegetative progeny of any cell are predominantly of the same type. Secondly, in such clones, cells occasionally arise that produce progeny of one type only; their instability has been lost; mating type is now apparently strictly inherited. The type of these stable lines, in the small number studied, was always the same as the type of the clone from which the unstable clone arose at endomixis (or autogamy), thus suggesting internal determination of the stable type.

We turn now to a class of hereditary intraclonal diversities observed by a number of investigators. These all involve numerical or architectural changes in self-reproducing parts of the cell. The simplest kind, found by Woodruff (31) in *Paramecium bursaria*, by Manwell (18) in *Pleurotricha*, and by others, consists simply in change of the number of nuclei per cell, and probably arose from unequal distribution of nuclei at fission. Another kind involves the production of normal single lines of descent from clones of double animals. This was found in *Oxytricha* by Dawson (4), in *Uroleptus* by Calkins (3), in *Colpidium* by Sonneborn (23), and in other genera by others. The single animals arose by a splitting apart of the two components, or by the passage of a normal fission plane across a cleft in the anterior part of the body. These single animals reproduce true to type, as the double animals ordinarily do. Finally, the reverse phenomenon has been described: lines of double animals may arise in clones of single animals. This may happen either by a direct fusion of two animals, as in *Oxytricha*, or by their origin from multiple monsters, as in *Colpidium*. In the latter case, they arise from regions of the monster in which two sets of organelles lie parallel and similarly oriented. In all these observations there is no indication of any change in the deep seated constitution of the cells; for the single animals that arise mechanically from doubles do not again produce doubles. Inheritance of these diversities thus appears comparable to the inheritance of chromosomal duplications or tetraploidy: rearrangements or changes in number of these structures are inherited because the structures are themselves self-reproducing.

Critical examination of the studies on inheritance during

vegetative reproduction in ciliates thus leads to the conclusion that nearly all the observations held to demonstrate the origin of hereditary variations within a clone are unconvincing. Of the apparently unobjectionable observations, most involve only a relatively superficial change in number or arrangement of non-genic but self-reproducing structures. Only two observations—the positive effects of selection in *Stylonychia* and the frequent change of hereditary characters in unstable clones of *Paramecium aurelia*—stand out as strong evidence of changes in basic constitution within a clone. As the members of a clone ordinarily must be alike in their genes, the question raised by these two studies is whether genic changes are occurring within the clone, or whether non-genic hereditary diversities are involved. The unique position of these observations in bearing on so important a matter is a clear call for further investigation of them if we are to attain any deeper insight into this fundamental problem of heredity during vegetative reproduction.

Let us turn now to heredity in sexual reproduction where fertilization and presumably, as in higher organisms, recombinations of chromosomes and genes should be involved. Much earlier work started by Jennings (9) on conjugation and by Erdmann (7) on endomixis showed that hereditary diversities arose at these processes. Recent studies (26, 27) on *Paramecium* have shown that such diversities are sometimes due to diversities in genes, as was generally supposed.

The most fully studied case (26, 27) involves a single locus in *Paramecium aurelia*. Individuals with only the recessive allele are always of mating type I and never can produce type II. Individuals containing the dominant allele may yield progeny of either mating type at autogamy. The characters determined by these alleles show typical Mendelian inheritance: dominants by recessives give heterozygotes; the F<sub>2</sub> gives the typical 1:2:1 ratio; and the back-cross, the 1:1 ratio. In all these crosses the two members of a pair of conjugants emerge from conjugation with identical genotypes, showing that the two pronuclei formed at the third maturation division are identical and that reduction must have occurred prior to this.

At autogamy (28) no genic recombination can take place in homozygous clones; but heterozygous clones are transformed invariably into homozygous ones; one-half becoming recessive and one-half dominant. This must mean that fertilization takes

place between the products of a single reduced nucleus. In view of Diller's (6) cytological observations, the alternative possibility—doubling of chromosome number in a single reduced nucleus—seems excluded.

If the behavior of this pair of genes turns out to be typical for ciliates, it will mean that once an individual has been through autogamy, its descendants can give rise to no other genic combinations until they conjugate with a genically different strain, or until mutation occurs. This is presumably the basis of Jollos' (13) observation that recombinations occur at conjugation only in stocks recently isolated from nature; for in nature crosses may occur and give heterozygotes, and when these are brought into the laboratory and cultivated as pure lines they quickly become homozygous.

The view of Jennings (12) and others that conjugation yields more variation than autogamy (endomixis) also receives some support from the newer knowledge of genic inheritance. Although in homozygous stocks neither conjugation nor autogamy can give genic variations, in heterozygous stocks autogamy yields only homozygotes while conjugation yields in addition heterozygotes. Conjugation would thus give, for genes showing dominance, a greater number of genotypes; and, for genes not showing dominance, both more genotypes and more phenotypes. The difference in results would increase rapidly with the number of genic pairs present in heterozygous condition.

There are some hereditary diversities recently studied (27, 28) in *Paramecium* that are difficult to interpret as due to genic differences. The most fully investigated is the diversity between the mating types in the stocks of *Paramecium aurelia* that contain both mating types I and II. The basic fact here is that the two individuals produced by the first division of an exconjugant or an exautogamous individual, may be different in mating type and give rise to clones that remain hereditarily diverse in mating type. If, as is believed, the micronuclei in these two clones are all derived by equational mitotic divisions from the same syncaryon they must be genically alike and cannot differ in the two mating types. However, the new genetic facts raise the question of whether, after all, the first and second post-zygotic micronuclear divisions are indeed equational. Genetically, this can be investigated by the standard procedure of comparing the progeny of the two diverse mating types to see



if they are diverse, as would be expected if the parents are genically different. When the test is made (25), by comparing the results of self-fertilization in the two types, it appears that they yield identical sets of progeny. At autogamy, each mating type produces both mating types and in the same proportions. Further, five successive autogamies in clones of type I produced at each autogamy the same results as in the preceding ones and the same as in five successive autogamies in clones of type II. The conclusion that the micronuclei in the two mating types in such stocks are genically identical seems inescapable.

There remains the possibility that the macronuclei in the two mating types are genically diverse. Again, present knowledge weighs against this, for the two new macronuclei produced in each exconjugant or post-autogamous individual are derived from divisions of the same syncaryon, presumably by equational mitoses. However, there could conceivably be a reduction or elimination of chromosomes in the development of macronuclei from micronuclei. In agreement with this, cytologists have reported extrusion of chromatin during this development. Although I at first was inclined toward this view, subsequent genetic evidence has led me to abandon it. For in the cross of homozygous stocks differing in the genes determining whether only type I or both types may be produced, the heterozygotes should give very different results depending upon whether the macronuclei are haploid or diploid. If they are haploid, the half of the clones that get macronuclei with the recessive allele can produce only type I, while the other half will produce some clones of each type, and this should result in an excess of type I among the hybrids; whereas, if all are diploid, no such restriction to type I exists and an excess of type I is not required. Actually, groups of hybrids sometimes produce the greatest excess of type II ever found, 75 to 85% of the resulting clones being of this type. Reduction of the genes apparently does not take place: the macronuclei remain diploid.

Conceivably other differences between the macronuclei might determine the difference between the two mating types. The macronuclei might differ in a particular translocation or inversion with the difference in mating type a position effect; but the high frequency with which this would have to occur, its limitation to a special moment in the life history, and its

dependence on the genotype make this possibility highly improbable. On the other hand, the dominant allele that permits development of either mating type may be an unstable gene mutating only in nuclei destined to develop into macronuclei and with a high frequency. Such mutations might occur in one of three ways. First, they could be from an indifferent to a differentiated condition (determining only type I or only type II). Second, they could be from either type to either type. If so, the mutations would have to occur at the same frequency in either direction, for the mating type ratio is independent of the parental type. Moreover, the assumption of an unstable gene for type II must be made in the face of evidence against the existence of any stable gene for this type. Finally, the observed effect of temperature set forth below would necessitate opposite temperature effects on the mutation rates of the two assumed alleles. The third possibility, that the micronuclear genes are always for one type and mutate only in the anlage of the macronucleus, would involve the enormous mutation rates of 50 to 85% if the micronuclear gene is always for type I and 15 to 50% if they are always for type II. While this last possibility is the least improbable, all these attempts to interpret the difference between the mating types as due to macronuclear differences of an orthodox genetic kind involve more or less improbable assumptions.

Could cytoplasmic differences, like plastid differences in some plants, be involved in the inheritance of these characters? The earlier mentioned phenomena of cytoplasmic lag indicate that cytoplasmic differences may determine mating type for a number of fissions.

On the other hand, much evidence shows the macronucleus, not the cytoplasm, is the determinant of mating types in *P. aurelia*. For the types change only when the macronuclei are destroyed and new ones are formed, i. e., at conjugation and autogamy. As the micronuclei are also undergoing special activities at the time the macronuclei are reforming and segregating, the question might be raised as to whether the mating types are directly determined by the micronuclei, not the macronuclei. Three lines of evidence together effectively eliminate this possibility. First, two cells produced by division of one differ in mating type only when two independently formed macronuclei separate, instead of dividing; this takes place normally at the

first fission after conjugation or autogamy when two new macronuclei are formed; at the second or third fission in those rarer cases when more than two new macronuclei are formed. Second, races have been found lacking micronuclei and yet with definite mating types. Third, as will be presently described, in some lines of descent new macronuclei develop from fragments of the old one: in these reorganizations the mating type never changes, in spite of the fact that micronuclear processes, possibly even syngamy, sometimes occur. In view of these facts, the mating types cannot be directly determined by the micronucleus, but must be directly under control of the macronucleus.

This macronuclear determination is affected by the temperature prevailing during conjugation or autogamy and the immediately following reorganization. The temperature effect is especially marked in variety 3, where the proportion of clones of type VI increases with temperature, though a similar relation appears to hold also in variety 1. The environmental conditions prevailing during nuclear reorganization thus appear to influence the character that will be determined by the new macronuclei. It is possible or even probable that the environment acts indirectly through the mediation of the cytoplasm, for at very low temperatures the types V and VI are not changed, both clones from each parent conjugant retaining the type of the parent, as occurs commonly at all temperatures in some crosses of types III and IV. In the inheritance of types I and II, the effect of the environment on the macronucleus—regardless of whether it operates through the cytoplasm or not—is confined to a brief sensitive developmental period and persists during vegetative reproduction.

The problem is therefore narrowed to this: the difference between the two mating types is due to a difference in their macronuclei and this is influenced by the environmental conditions prevailing at the time the macronuclei develop from micronuclei and at no later time; how then do the macronuclei differ? In the preceding discussion the improbability of any genic difference was pointed out, but no more probable interpretation is apparent.

A very different kind of inheritance has just come to light in my current studies on *P. aurelia*. A previously undescribed method of nuclear reorganization has been found both in single animals, like autogamy, and in paired animals, like con-

jugation. The micronuclei may or may not be present, but they do not give rise to macronuclei. Instead, new macronuclei develop from the fragments of the old macronucleus which has disintegrated, as in conjugation and autogamy, by unraveling into skeins and breaking up into spherical pieces. Each of the many spherical fragments develops into a new macronucleus and these are segregated at subsequent fissions until only one is present in each individual. This shows that the macronucleus is compound, for each fragment contains all that is required for normal macronuclear functioning.

The mating type of an animal never changes at this process of macronuclear regeneration, in contrast to the regular changes occurring at normal autogamy and conjugation. On the other hand, a whole complex of new characters always appears whenever macronuclear regeneration has occurred. The animals become smaller and of different form; they reproduce more slowly; they are less viable and die out after a period of weeks or months; new reorganizations of the same kind follow at very short intervals; the mating reaction is unusually intense; and the micronuclei often disappear. This new reorganization process and its regular consequences have been observed in several races; in certain lines of descent it is the only reorganization process that occurs. We have here therefore a definite complex of hereditary characters repeatedly appearing as a consequence of a simple macronuclear regeneration, presumably involving no genic change whatever. I find in the literature a number of accounts of this same complex of characters appearing under conditions interpreted as frequent mutations or genetic variation at endomixis; but it now appears that these may be misinterpretations resulting from inadequate knowledge of the cytological processes involved. What we have essentially is a reorganization that is purely vegetative, not involving the formation of genetically different macronuclei or of new clones, but illustrating hereditary diversity within what appears to be a genically uniform clone.

A number of observations in the literature, viewed in relation to the newer work just set forth, will illustrate further the difficulties involved in attempting to extend to such situations in the ciliates the principles of genetics that apply so generally to higher organisms.

After long-continued inbreeding, races studied by Jennings (9) continued to give hereditary variations at conjugation. His suggestion that Mendelian recombinations might not be the whole secret of the matter is now strengthened; for our present knowledge of the genetic consequences of autogamy makes it practically certain that his races did quickly become homozygous for all their genes. Similarly, the paramecia used in the later studies of Raffel (21), of Sonneborn and Lynch (29) and of others were also probably homozygous. It would appear therefore that the many hereditary diversities found in all these studies must have been due either to mutations or to some non-genic differences.

Another class of observations includes the frequent exceptions to Jennings' principle of biparental inheritance. Jennings (9) discovered long ago that after two individuals conjugate they tend to produce similar clones. He designated this relation as biparental inheritance. The similarity of the two exconjugant clones was interpreted as indicating they had identical genes; and the identity in genes was the result of the formation of the gamete nuclei from a haploid nucleus produced by chromosome reduction at the first two maturation divisions. The recent work (26, 27, 28) on genic inheritance in *P. aurelia* is a striking proof of the correctness of this view.

Many studies have shown, however, that in certain characters the two conjugants of a pair frequently give rise to diverse clones. This has again been confirmed recently: Jennings (10, 11) found it in about  $\frac{1}{8}$  of the mate-pairs in *P. bursaria*; I (27) have found that mating types in *P. aurelia* are invariably diverse in the progeny of mate pairs in certain crosses and under certain conditions, while under other conditions the same characters show similarity in the two mate-progenies no more often than expected by chance. Applying the same reasoning as before and still assuming genic determination of the characters, some students (22) of the problem have suggested or concluded that differences between the progeny of mates must be due to differences between their gamete nuclei resulting from reduction at the *third* maturation division. In *P. aurelia* evidence I have previously set forth shows that the known gene pair is always reduced prior to this. As there is at present no proof of third division reduction in any case and

as the evidence demonstrates earlier reduction in the only known case, it would now appear that the weight of existing evidence points not to third division reduction, but to some other cause as the usual basis of differences between mate progenies. Whether this other cause is again mutation or some non-genic mechanism cannot at present be decided.\*

There remains for consideration one great class of observations in ciliate genetics. Jollos (13), in one of the most intensive and extensive series of studies in ciliate literature, arrived at the conclusion that many characters persisting through hundreds of fissions and even sometimes through autogamies (endomixes) and conjugation were not genic but due to the long-lasting effects of environmental conditions on the plasma, effects that always eventually wear off and disappear under action of the unaltered genes. This class of characters he calls "Dauermodifikationen." In some respects these phenomena are similar to the inheritance of mating types in stocks of variety 1 of *P. aurelia* that produce both of them. In both cases, the hereditary differences may not be due to genic differences. In both, the same environmental conditions determine the production of the characters in some races but not in others. Finally, the mating types, like Dauermodifikationen, may change at autogamy and conjugation.

But in other respects, the mating types differ radically from Dauermodifikationen, as portrayed by Jollos. Dauermodifikationen are producible within a clone, presumably at any time, while mating type changes occur only at nuclear reorganizations. Dauermodifikationen disappear eventually during the passage of fissions alone, and gradually, in a series of step-like stages; mating types persist indefinitely during fissions and normally change only at reorganizations and not in step-like gradations. Dauermodifikationen may persist through several nuclear reorganizations in all lines of descent before they disappear in all; mating types never show this behavior. Change

\* Genetic experiments performed by the author since this paper was presented have shown that in *P. aurelia* some of these differences between mate progenies are due to the occasional failure of conjugants to exchange gamete nuclei, in agreement with previous cytological observations on other species of *Paramecium* by Diller and by Wichterman. However, it seems unlikely that all such differences will be found to be due to this, for many have been observed in conjugation between presumably identical homozygotes.

of Dauermodifikationen is accelerated by frequent change of environmental conditions; there is no evidence that anything but nuclear reorganization can normally change mating types.

Dauermodifikationen are interpreted by Jollos as plasmatic and not genic, though others (21) have attempted to interpret them as gene mutations. It would be desirable to reinvestigate these phenomena employing the methods of genic analysis now available; but Jollos (14) has already pointed out how highly improbable the mutation interpretation is.

I have reviewed in this paper some situations in ciliate genetics in which typical genic inheritance is unquestionably involved; but I have also attempted to set forth a number of situations, including by far the greatest part of what is known about ciliate genetics, which are extremely difficult to interpret along orthodox genic lines: the existence of hereditary differences within a clone; the inheritance of mating type in stocks of *Paramecium aurelia* that contain both types I and II; the inheritance of the complex of characters associated with macronuclear regeneration; the failure to obtain by inbreeding clones that remain constant at conjugation; the production of hereditary diversities of many kinds at conjugation and autogamy in material that should be homozygous; the production of diverse progenies from two individuals that have conjugated with each other and should be genically alike; and the great class of Dauermodifikationen presumed to be plasmatically determined. Attempts have been made, notably by Raffel, to interpret many of these phenomena in terms of mutations; similar interpretations can be made for all of them. Such interpretations are however purely formal and there is no genetic situation, however bizarre it may be, that cannot be "explained" by mutations if appropriate assumptions are made. To treat all the phenomena discussed here in this way would require many and often highly improbable assumptions. Yet these may turn out to be justified. They may be no more remarkable than the macronucleus, uniquely confined to ciliates, which may present especially in its early development conditions highly favorable to mutation. On the other hand, as Jollos maintains, non-genic factors may be involved. One of the main tasks students of the genetics of ciliates face is the solution of this difficult but important problem.

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# The Physico-chemical Properties of the Nucleus

*By*

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## INTRODUCTION

It is a commonplace that the unit structure of life, the cell, embodies in its own protoplasm another structure, the nucleus. Indeed, the very definition of a cell involves the concept of a mass of protoplasm containing a nucleus. To the cytologist, then, the nucleus is a well-known morphological entity. For the physiologist, however, the nucleus remains more or less of an enigma.

The reasons for this state of affairs are not hard to find. In addition to the difficulties attending any investigation of objects of the minute dimensions of living cells, one is confronted with the fact that the nucleus cannot be approached directly by experimental means. Surrounded by cytoplasm, the nucleus reacts in part to the experimental reagent itself, and in part to secondary effects resulting from the reactions of the experimental reagent upon the cytoplasm. True, modern techniques permit one to work on isolated nuclei (see, e.g., Stoneburg, 1939). It is a question, however, in how far such nuclei may be considered normal. The impossibility of ever really succeeding in eliminating, or even correctly evaluating, secondary cytoplasmic effects should be borne in mind continuously by the investigator.

The purpose of this paper is to review briefly some of our information on the physico-chemical properties of the nucleus. Obviously, because of limitations of space, the treatment will not be exhaustive. Many subjects (such as pH, specific gravity, metabolism, chemistry of the chromosomes—all of which have large literatures) will have to be neglected. In the main I have selected topics for discussion with which I have had direct

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experience. I propose to discuss the following five topics in the order given: (A) colloid chemical properties of the nucleus and of the cytosome, (B) viscosity, (C) osmotic properties of the nucleus, (D) permeability of the nuclear membrane to water, and, (E) amphoteric properties of the nuclear constituents.

### A. COLLOID CHEMICAL PROPERTIES OF THE NUCLEUS AND OF THE CYTOSOME

A prime characteristic of protoplasm is its tendency to form vacuoles. A thorough discussion of the universality of this reaction and the mechanism involved will be found in Chapter 14 of Heilbrunn's *Colloid Chemistry of Protoplasm*, 1928. The ability of naked protoplasm to form rigid membranes is seen not only in such a phenomenon as cytolysis, but also in the case of repair of the cell membrane on injury. Heilbrunn and others have shown that the calcium ion is necessary for the reaction and that the mechanism of film formation is apparently concerned with the formation of a calcium proteinate type of compound possessing rigid, gel-like properties. Of course vacuolization, cytolysis, hemolysis, and repair of cell surface are phenomena associated with the cytoplasm and the cell membrane. It is only fair to ask, Is the protoplasm of the nucleus endowed with similar properties?

It may be stated definitely that, in so far as my own experience goes, the nucleoplasm and the nuclear membrane are incapable of forming new membranes under the conditions suitable for such formations by the cytoplasm. Materials studied include the nuclei of marine ova, of grasshopper spermatocytes, of insect salivary gland cells, of insect gut cells, of single muscle fibers, and of insect ganglia. When the nucleus is crushed in a Ringer's solution or in an isotonic calcium chloride solution, there is no vacuole formation. The broken nuclear membrane has the appearance of a collapsed sac with no evidence of attempted repair.

An experiment illustrating the inability of the nucleus to show a "surface precipitation reaction" is as follows: Grasshopper spermatocytes are dissected in an isotonic  $\text{CaCl}_2$  solution. In the resting stage the cytoplasm of these cells contains mitochondria in the form of granules (Lewis and Robertson,

1916). Immersion in an isotonic  $\text{CaCl}_2$  solution causes vacuolization of these mitochondria. No vacuoles are to be seen in the nucleus. It might be argued that the calcium ion cannot penetrate the nuclear membrane as it does the cell membrane. But even when the cells are crushed in this solution, vacuoles do not form in the nucleoplasm.

A clue to an explanation of the difference in behavior between the protoplasts of the cytosome and of the nucleus is to be sought, perhaps, in the following facts. The cell membrane is generally negatively charged, whereas the nuclear membrane bears a positive charge. It will be shown further on that the pH of the isoelectric point of the nuclear membrane is high; so high, indeed, that one may safely assert that the membrane exists as a protein-anion complex, the protein acting as base. Now the nuclear membrane, lying as it does on the acid side of its isoelectric point, cannot combine with cations like calcium. Since the property of film formation, however, appears to depend upon the establishment of a calcium proteinate, it is obvious that the nuclear membrane—existing as a protein-anion combination—cannot possibly possess this physiological property of film formation.

There is one structure in the nucleus capable of showing vacuolization. This is the nucleolus. As early as 1864, Balbiani noticed vacuolated nucleoli in the ova of *Geophilus longicornis*. I have observed the formation, in the living, of vacuoles in the nucleoli of the oocytes of *Arbacia* and *Nereis*, as well as in the large nucleolus of the fourth chromosome of the salivary gland cells of *Chironomus*. This latter observation has been recorded many times (van Herwerden, 1910; Vonwiller and Audova, 1933; Bauer, 1935). It is not at all certain, however, that the reaction in the nucleolus is comparable to that in the cytoplasm.

Conclusion: In this section it has been shown that the protoplasm of the nucleus and that of the cytosome have certain features in common, but differ in many respects too. For example, both the nuclear and the cell membrane are semipermeable. On the other hand, the nucleoplasm and the nuclear membrane are incapable of forming films in the presence of free calcium ions—a property common to almost all kinds of cytoplasm. The cause for the differences in behavior of these

two types of protoplasm is probably to be sought in the amphoteric nature of their constituents; the latter most likely being identifiable with the proteins.

## B. VISCOSITY

One of the most satisfactory means of describing the physical state of protoplasm is in terms of its viscosity. The viscosity of the nuclear sap has been the object of but few investigations (Heilbrunn, 1928, pp. 83-85). Quantitative determinations of the viscosity of the nucleoplasm have been made by two methods: (1) the application of Stokes' law to the case of the nucleolus of the *Echinus* oocyte falling freely through the fluid protoplasm of the germinal vesicle under the influence of gravity (Heilbrunn, 1928; Harris, 1939); and (2) observation on Brownian motion (Harris, 1939).

Using Gray's (1927) data for the velocity of fall of the nucleolus, and assuming the difference in specific gravity between the nucleolus and the nucleoplasm to be 0.1, Heilbrunn estimated the viscosity of the nuclear fluid as twice that of water (see, however, Harris, 1939, p. 271). Harris, introducing the Ladenburg correction for the fact that the nucleolus does not fall through a medium of infinite extent, arrived at a figure of 0.101 poises. By studying the Brownian movement of minute particles in the germinal vesicle, Harris estimated the viscosity as 0.094 poises. That these estimates are reliable only in so far as they give the order of magnitude of the viscosity of the nucleoplasm is fully realized by Harris. He states (p. 271), "The closeness of the agreement is of course quite fortuitous; the error of the Brownian movement may well approach  $\pm 30\%$ , while the other value is based on a purely hypothetical estimate of the specific gravities of the nucleolus and the nuclear fluid."

Of even greater interest is the observation of Harris that "the nuclear fluid is thixotropic." He found that "In a series of successive falls, the maximum velocity attained during the initial fall is significantly less than that of later falls." Also, "complete restitution of its elastic structure takes place within 10 min. of its disruption by the moving nucleus." In other words, the viscosity of the nucleoplasm varies with its mechanical his-

tory, and the coefficient of viscosity is not independent of the velocity gradient.

Conclusion: There is singular significance in the fact that the nucleoplasm behaves as a non-Newtonian fluid, possessing thixotropic properties. For it is the general opinion of cellular physiologists that the physical basis for the physiological activities of protoplasm is to be found in the ability of the latter to undergo reversible sol-gel transformations. And according to Clayton (1935, pp. 125-126), it is precisely those fluids that possess this "structural viscosity" shown by the nucleoplasm that are capable of reversible solation and gelation.

### C. OSMOTIC PROPERTIES OF THE NUCLEUS

Although extensive investigations have been carried out upon the osmotic behavior of cells and tissues, comparatively little attention has been devoted to the study of the osmotic properties of the nucleus. To be sure, there are numerous observations of a qualitative nature on reversible swelling and shrinking of nuclei, the most comprehensive of which are probably those of Shinke (1937). He reported that the nuclei of some 20 species of plant cells, as well as the nuclei of numerous animal cells, react osmotically.

Investigations of a more quantitative nature were conducted by Hamburger (1904) on the osmotic behavior of the nuclei of gut epithelium in anisotonic NaCl solutions, and by Skowron and Skowron (1926) on the behavior of the germinal vesicle of the egg of *Sphaerechinus* in hypotonic dextrose solutions. In addition the latter investigators found that, although the *Sphaerechinus* egg shrinks in hypertonic solutions, the nucleus does not. This observation can only be interpreted as meaning that the nucleus does not behave osmotically; a conclusion at variance with my own observations on the osmotic properties of the germinal vesicles of *Arbacia* and *Nereis* ova.

According to the Boyle-van't Hoff law, the pressure-volume products of the nuclei in 100% sea water ( $P_0V_0$ ) must equal their pressure-volume products at equilibrium in anisotonic solutions ( $P_1V_1$ ). Data for the germinal vesicle of the egg of *Sphaerechinus granularis* (calculated from the figures of Skowron and Skowron) are presented in Table 1a. Data for the germinal vesicle of the *Arbacia* oocyte are given in Table 1b,

and data for the germinal vesicle of the egg of *Nereis limbata* are to be found in Tables 2a, 2b. In Table 1a,  $P_0V_0 = 9.74$ , and the other figures in the column " $\Delta T.V_1$ " are  $P_1V_1$ . In my own tables the values of  $P_0V_0$  and  $P_1V_1$  are given directly. In Tables 1b and 2a the values of the pressure-volume products

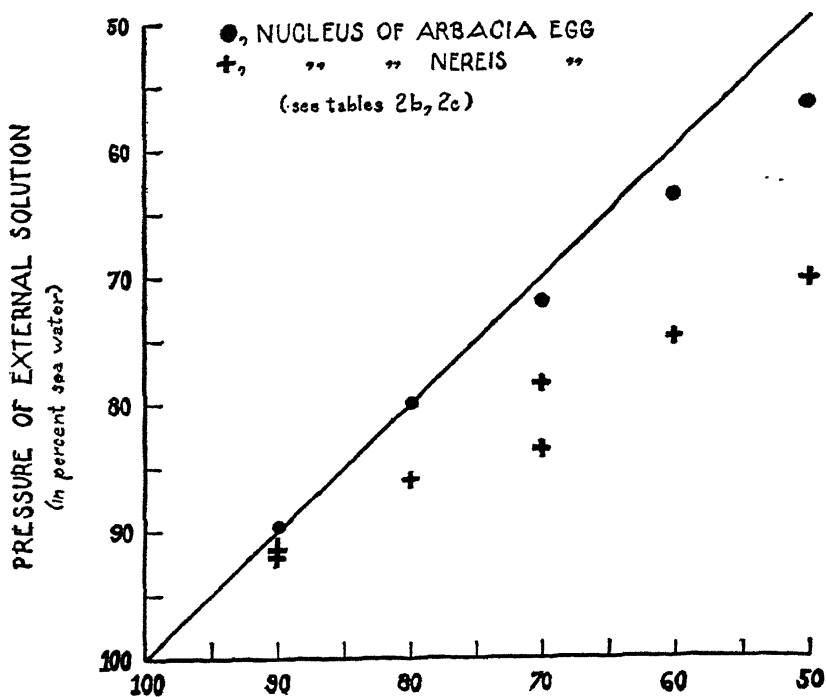


FIG. 1 PRESSURE WITHIN THE NUCLEUS - CALCULATED  
(in percent sea water)

of the nuclei on return to 100% sea water are also given ( $P_0V_0$ ).

The results indicate (1) that the nuclear membrane is semi-permeable (compare the figures under " $P_0V_0$ " with those under " $P_0V_0''$ "); and (2) that with increasing dilution there is an ever-increasing deviation from the Boyle-van't Hoff law (compare the data for " $P_0V_0$ " with those for " $P_1V_1$ ," as well as the figures in the column "Percent Deviation").

Another way of showing this deviation is seen in Fig. 1, where the values of the actual external pressures—in percent



sea water—are plotted against the pressures existing within the nuclei at equilibrium, calculated from the relation,

$$P_1 = \frac{P_0 V_0}{V_1}$$

The heavy diagonal line represents the pressures that *should* exist within the nuclei if the latter were perfect osmometers.

It is not possible to discuss the causes of these discrepancies in this paper, although I may point out that when the values of the non-osmotically active material are calculated for successive dilutions, they are found to vary with the concentration. Also, it can be seen (Table 1b) that the nuclei in 90% and 80% sea water give no evidence of having any significant amount of osmotically inert material.

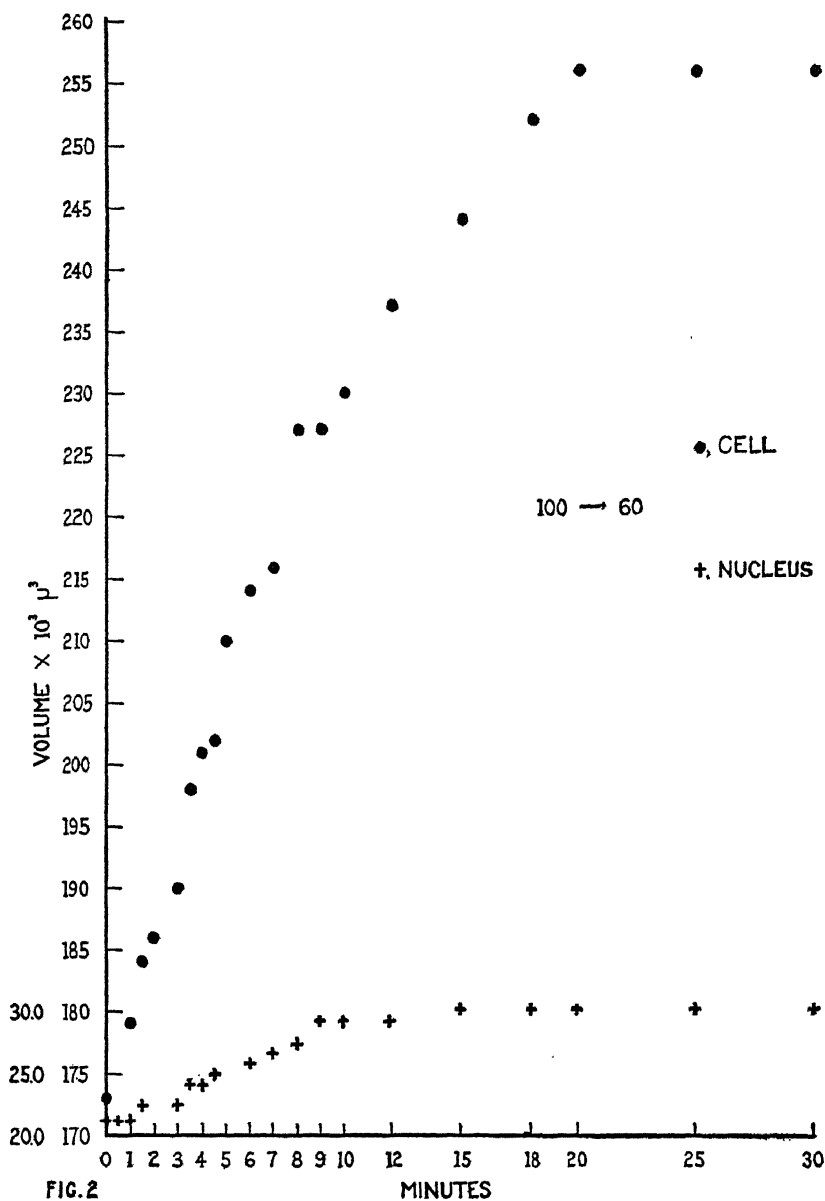
Finally, it is interesting to note that the nucleolus acts osmotically. Three *Arbacia* eggs whose nucleoli each had an initial volume of  $0.8 \times 10^3 \mu^3$  in 100% sea water were transferred to 60% sea water. After a  $\frac{1}{2}$  hour in this solution, two of the nucleoli had attained the volume of  $1.3 \times 10^3 \mu^3$ , and the other,  $1.6 \times 10^3 \mu^3$ . A  $\frac{1}{2}$  hour after restoration to 100% sea water, two of the nucleoli had returned to their original volumes of  $0.8 \times 10^3 \mu^3$ , and the third had a volume of  $0.9 \times 10^3 \mu^3$ .

#### D. PERMEABILITY OF THE NUCLEAR MEMBRANE TO WATER

In the preceding section I have discussed the application to the nucleus of the laws of osmotic equilibrium. One may also inquire into the kinetics of water penetration. A curve for the rate of entrance of water into the germinal vesicle of the starfish egg has been plotted by Beck and Shapiro (1936), and one for that of the *Ceratocephale* egg by Kamada (1936).

Typical swelling curves for the cell and germinal vesicle of *Arbacia punctulata* in 60% sea water are presented in Fig. 2. Curves similar to these were plotted for various anisotonic solutions, and the permeability coefficients determined from the integrated equation of Lucké and McCutcheon (1932, p. 105).

Before presenting the actual data it should be noted that, in applying the diffusion equation to the case of the nucleus, a



further assumption is necessary. The equation assumes that the volume of the external solution is infinite with respect to that of the cell; i.e., that the external concentration does not change during the experiment. Obviously the nucleus is not in direct contact with the external medium. Strictly speaking, its water content is determined by the ever varying concentration in the cytosome. However, as a first approximation one may regard the concentration gradient as existing between the external solution and the nucleus. This assumption will be valid if the permeability coefficients during the course of an experiment are constant.

Actually, the values of  $k_c$  and  $k_n$  (expressed as cubic micra of water traversing one square micron of cell or nuclear surface per minute per difference in osmotic pressure of one atmosphere) are fairly constant. Values of the permeability coefficients for the cell and nucleus in Fig. 2, typical of other experiments, are given in Table 3.

TABLE 3  
*ARBACIA PUNCTULATA*

Time (min.)	$k_c$	$k_n$
1 .....	0.06	
1½ .....	0.08	0.04
2 .....	0.07	
3 .....	0.06	0.02
3½ .....	0.08	0.04
4 .....	0.08	0.04
4½ .....	0.07	0.05
5 .....	0.09	
6 .....	0.09	0.05
7 .....	0.08	0.05
8 .....	0.10	0.06
Average 0.08		Average 0.04

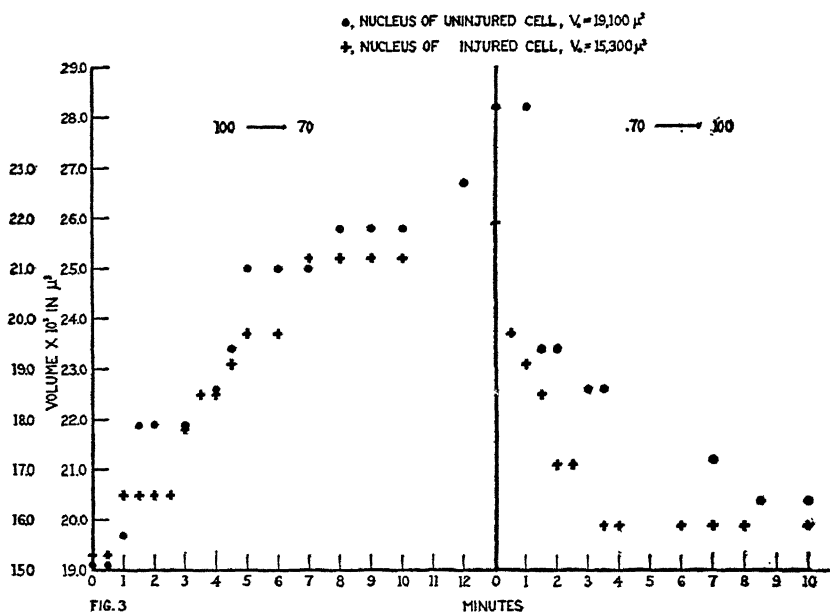
It is also possible to test experimentally the assumption that the driving force is between the external solution and the nucleus. Lucké and McCutcheon have shown (1932) that the cell becomes freely permeable upon injury. Accordingly, cells were injured mechanically and the rate of swelling and shrink-

ing of the nuclei compared with that of nuclei in uninjured cells (Fig. 3). There were no significant differences (Table 4).

TABLE 4  
*ARBACIA PUNCTULATA*

Nucleus of uninjured cell				Nucleus of injured cell			
k (swelling)		k (shrinking)		k (swelling)		k (shrinking)	
Number	Average	Number	Average	Number	Average	Number	Average
12	0.06	6	0.07	10	0.06	5	0.08

Theoretically, the permeability coefficients should be independent of the salt concentration of the medium. The values of  $k_e$  and  $k_n$  in other anisotonic solutions are similar to those in 60% sea water, although the values in passing from 131%



and 113% sea water to 100% sea water are slightly less. There is no evidence of a drift.

TABLE 5

SWELLING OF CELLS AND GERMINAL VESICLES OF *ARBACIA PUNCTULATA* IN 60% SEA WATER

Cells			Nuclei		
Initial Volume $\times 10^3 \mu^3$	Number of ks	Average k	Initial Volume $\times 10^3 \mu^3$	Number of ks	Average k
114	10	0.14	15.9	8	0.09
158	5	0.12	19.1	12	0.06
173	15	0.09	21.2	10	0.05
157	7	0.08	20.4	4	0.03
170	6	0.14	19.7	9	0.07
150	2	0.12	19.7	8	0.04

The biological variations from cell to cell and nucleus to nucleus in any one concentration may be high (see, e.g., Table 5). It must be remembered that these immature ova are quite different physiologically at different times during the season.

During the course of these investigations two mature ova were encountered which possessed extraordinarily large nuclei. (These cells were perfectly normal, as was verified by fertilizing them and noting that they cleaved perfectly.) The nucleus of one had an initial volume of  $2,000 \mu^3$ ; the other,  $9,300 \mu^3$ . The average value of  $k_n$  during swelling was 0.02.

#### Summary:

1). The permeabilities to water of the cell and germinal vesicle of *Arbacia punctulata* obey in general the diffusion laws.

2). The permeability to water of the immature egg is the same as that of the mature, unfertilized egg; i.e., there is no change in permeability to water upon maturation.

3). The permeability coefficients for cell and nucleus are constant during the course of any single swelling experiment. Roughly, the value of  $k_c$  is 0.1; of  $k_n$ , 0.05. There is considerable variation from experiment to experiment.

4). The nuclei of uninjured, and of mechanically injured, ova have similar permeability coefficients.

5). The permeability coefficients appear to be greater during shrinking than during swelling.

6). The permeability coefficients are independent of the salt concentration of the medium.

- 7). The permeability coefficients do not appear to be strictly correlated with the initial volumes.
- 8). The average value of  $k_n$  for extraordinarily large nuclei in normal, mature, unfertilized ova was 0.02.

### E. AMPHOTERIC PROPERTIES OF THE NUCLEAR CONSTITUENTS

The various constituents of protoplasm, being suspensoids or colloids, have an electric charge. The sign of the charge of the chromosomes has been the subject of numerous investigations. It is generally agreed that the chromosomes are negatively charged.

A fact about the nucleus much less generally known is that the nuclear membrane bears a positive charge. This was first discovered by Dahlgren (1915) for the nuclei of the motor nerve cells of the electric lobe of *Torpedo ocellata* (see especially his Fig. 19, and accompanying legend).

A preparation for studying the charge of chromosomes and nuclei in the living was described by Churney and Klein (1937), using the salivary gland cells of the larvae of *Sciara coprophila* and of *Chironomus sp.* The giant chromosomes and nucleoli are negatively charged; the nuclear membrane is positively charged. Recently I have been able to extend these findings for the nuclear constituents of single muscle fibers, salivary gland cells, ciliated gut cells, and ganglia, of the larva of *Chironomus*. Attempts were made to determine whether or not the light and dark bands of the giant chromosomes of the salivary gland cells of *Chironomus* react differentially in an electric field. No such effect was found.

It has long been known that the chromatin acts as an ampholyte. The principal evidence in favor of this view is derived from the observation that chromatin will stain with either acid or basic dyes, depending upon the pH of the medium (see Conn, 1936, p. 176). I attempted to determine cataphoretically the isoelectric point of the giant chromosomes and of the nuclear membrane of the salivary gland cells of *Sciara coprophila* larvae. As yet the results must be considered provisional. It is not possible to discuss here methods, necessary precautions, and sources of error. Unbuffered Ringer's solutions were used. The pHs were adjusted by adding small amounts of 0.105

N HCl and 0.119 N NaOH, thereby maintaining the isotonicity of the surrounding fluid.

The sign of the charge of the nuclear membrane and the chromosomes was determined using intact cells, isolated nuclei, and nuclei from which the chromosomes had been partially extruded by means of pressure. The isoelectric point of the chromosomes was found to lie between pH 3.3 and 3.6; that of the nuclear membrane between 10.6 and 11.2. (Note: the alkaline solutions pick up  $\text{CO}_2$  so readily that, even using special precautions, the isoelectric range cannot safely be narrowed down any further.)

Now it can be shown that the chromosomes normally exist at a pH close to neutrality. A rough way of demonstrating this is to use the chromatin itself as an indicator. In acid solutions the refractivity and opacity of the various parts of the chromosomes increase in a definitely graded fashion; in alkaline solutions the reverse holds. Moreover the chromosomes appear to be very sticky in acid solutions, whereas they do not adhere to the nuclear membrane or to chemically clean slides in neutral or alkaline solutions. At very high pHs the chromosome coils and bands cannot be distinguished as such. All that is seen is a faint central mass that appears to "flow" when the current is applied. (Isolated nuclei are actually invisible at pH 11.3, but become refractive on applying the current!)

Using these characteristics as criteria, it was found that the chromosomes in paraffin oil or body fluid preparations compared closely with those dissected in a solution of pH 7.2. It may be noted that the acid pH effects are reversible—passing from pH 7.2 to 3.0 and back to 7.2. The alkaline effects are not reversible. Chromosomes immersed in a solution of pH 11.3 and then transferred to a solution of pH 3.7 maintain the appearance of chromosomes in a highly alkaline medium.

The chromatin, lying on the alkaline side of its isoelectric point, should, like the serum proteins, bind cations strongly. The nuclear membrane, lying on the acid side of its isoelectric point, must exist as a protein-anion complex.

The fact that the nuclear membrane acts as an ampholyte with such a high isoelectric point leads one to believe that it must be constituted largely of the basic polypeptides. Of the basic amino acids (histidine, lysine, and arginine) only arginine, with its guanidine radical, has an isoelectric point at a com-

parable pH (see Schmidt, 1938, p. 613). Accordingly, attempts were made to adopt the specific Sakaguchi test for arginine (as modified by Weber, 1930) as a histochemical test. No satisfactory results were obtained, however.

## CONCLUSION

This paper has been concerned with some of the physico-chemical aspects of the nucleus. At this time, when the geneticist is becoming impatient to know the mechanisms of genic action, it is important to have accurate and quantitative data on the physics and chemistry of the system in which the gene is located—the chromosomes within the nucleus. It is hoped that at least one service that this paper may render is to indicate the great amount of work that remains to be done in this field.

TABLE 1A  
*SPHAERECHINUS GRANULARIS*  
(After Skowron and Skowron, 1926)

Number of Nuclei	Pressures $\Delta T$	Equilibrium Volumes	$\Delta T \cdot V_1$	Percent Deviation
14 .....	2.28	4.268	9.74	
7 .....	1.73	6.254	10.81	10.9
11 .....	1.70	6.954	11.82	21.2
12 .....	1.26	7.553	9.51	-2.4
10 .....	1.08	10.384	11.21	15.1
10 .....	0.97	6.018	5.84	-40.1

$\Delta T$ , freezing-point lowering in degrees Centigrade.

TABLE 1B  
*ARBACIA PUNCTULATA*

Number of Nuclei	Relative Pressures	$P_0 V_0$	$P_1 V_1$	Percent Deviation	Number of Nuclei	$P_0 V_0$	$P_0' V_0'$	Percent Deviation
6 .....	1.13	30.8	28.1	8.8	3 .....	34.0	32.0	-5.9
17 .....	0.9	20.5	20.4	-0.5	10 .....	20.5	20.4	-0.5
46 .....	0.8	27.5	27.5	0.0	13 .....	28.2	27.9	-1.1
51 .....	0.7	27.5	26.8	-2.5	24 .....	28.1	28.7	2.1
53 .....	0.6	28.0	26.4	-5.7	19 .....	27.5	27.5	0.0
48 .....	0.5	28.8	25.4	-11.8	8 .....	28.0	27.9	-0.4



TABLE 2A  
NEREIS LIMBATA

Relative Pres-sures	Number of Nuclei, $V_0$	Number of Nuclei, $V_1$	$P_0V_0$	$P_1V_1$	Percent Devia-tion	Number of Nuclei, $V_0'$	$P_0'V_0'$	Percent Devia-tion
0.9	56	64	98.37	96.34	- 2.06	27	95.90	-2.51
0.9	73	61	103.33	101.83	- 1.45	60	107.62	4.15
0.8	51	43	101.14	94.21	- 6.85	38	104.33	3.15
0.7	53	56	100.67	84.32	-16.24			
0.7	57	58	98.53	87.86	-10.83	20	95.77	-2.80
0.6	27	49	127.47	102.24	-19.78	51	123.82	-2.86
0.5	49	44	108.99	77.56	-28.83	35	116.63	7.01

TABLE 2B  
NEREIS LIMBATA

	$P_0V_0$	$P_1V_1$ in 80% sea water	Percent Deviation	$P_1'V_1'$ in 60% sea water	Percent Deviation
A.	90.17	90.34	0.19	78.67	-12.75
B.	91.71			81.52	-11.11

	$P_0V_0$	$P_1V_1$ in 70% sea water	Percent Deviation	$P_1'V_1'$ in 50% sea water	Percent Deviation
A.	96.44	81.95	-15.02	68.15	-29.33
B.	95.60			71.14	-25.58

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BICENTENNIAL CONFERENCE

# The Chromosomes of the Amphibian Nucleus

*By*

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THE focus of the nucleus problem is: How do chromosomes function? In spite of the immense cytological literature and the careful experiments of many geneticists we are still very much in the dark as to the actual means whereby certain chemical molecules control and organize other molecules into protoplasmic organ systems. As a main purpose this paper attempts to show that chromosomes in amphibian eggs genically produce materials for the embryo by a process of lateral synthesis. These synthetic materials are in the form of chromosomal side loops. Such loops are not to be confused with mere reduplication of chromoneme strands, as Painter and Koltzoff have recently done. On the contrary the lateral loops are products manufactured at definite homologous places in the chromosome cylinder, and must therefore be considered as of genic origin.

A comprehensive review of amphibian cytology is here out of the question. Moreover, excellent recent summaries are available in the papers of Nebel (1939) and Milovidov (1936). Particularly significant contributors to the problem in hand include Ransom (1867), Rückert (1892), Wagner (1923), Brachet (1929), Koltzoff (1938) and Gersch (1940). It is both a pleasure and a duty to acknowledge our debt to Professor Chambers, whose classical paper in 1914 on the cell nucleus and whose remarkable instruments have proved so useful. A reanalysis of chromosome structure has been accomplished utilizing, on the one hand, Chambers' micro methods, and on the other, this new material consisting of washed nuclei from amphibian eggs and of isolated chromosome pairs in physiological media. This is a fundamentally different approach from the older methods

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of fixing and sectioning eggs. I developed these techniques first in 1936, while at Professor Baltzer's laboratory in Bern, with the purpose of studying the function of a nucleus in the cell. Procedures included micro-dissection, micro-injection, and the systematic application of chemical reagents and physical forces such as X-rays, enzymes and dyes.

No consideration of chromosome structure would be meaningful, without constantly bearing in mind the colloidal factors of the normal complex nuclear medium. However, colloidal relationships involve too many details in themselves, so they must perforce be considered at some other time and place and will be published elsewhere.

In a fresh isolated germinal vesicle of a frog, the 13 pairs of chromosomes can be observed in their three-dimensional relationships. In *Rana temporaria*, for example, there are always 2 long pairs, 5 short pairs, 4 ring pairs and 2 abbreviated ones. In each the number of chiasmata appears constant. As Wagner showed in 1923, these pairs remain in synapsis for at least three and possibly for several more years. It should be recalled that in ovogonia the chromosomes start as small threads only a few micra in length and reach their maximum size in approximately the half-size egg, only to shrink back again during later egg growth, so that they may be ready to separate on the first maturation spindle. Data in this paper refer to the middle range. The longest chromosome in *Triturus pyrrhogaster* is at the maximum stage over 435 micra, shown in the accompanying photograph (Fig. 1). Another, somewhat stretched, measures 810 micra. Other isolated chromosomes, relaxed by raising the pH, followed by a mild acidification show elongated filaments considerably over a millimeter. The maximum normal extension of amphibian chromosomes places them in the category of the largest known, since they are approximately twice the length of so-called giant salivary gland chromosomes of the Diptera.

Each amphibian chromosome consists essentially of a single plastic cylinder, with imbedded granules and attached loops, as indicated in Fig. 2. Unusual elastic and mucoid properties of this cylinder allow great changes in length without visible coiling or uncoiling. Elasticity resembles that of a rubber band. It is unnecessary to debate the problems of coiling so fully described for other types of chromosomes, or to deny that coiling

FIG. 1

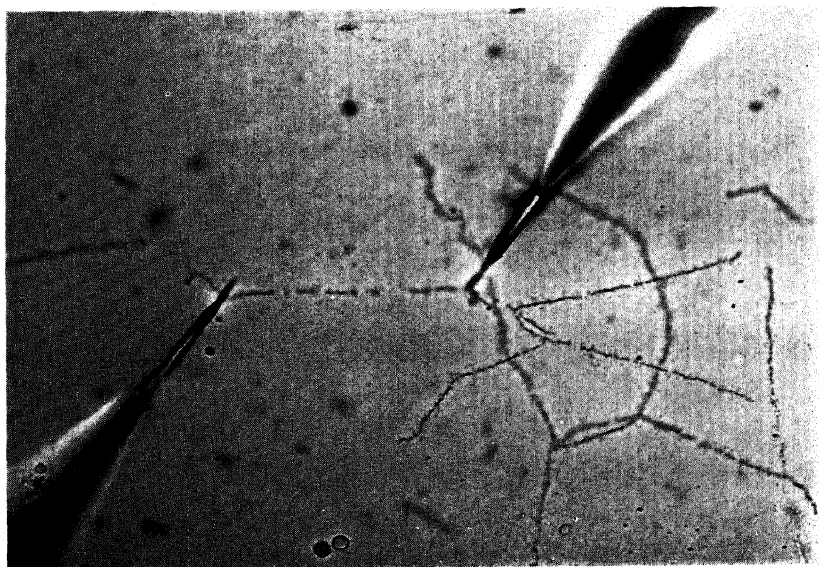


FIG. 3

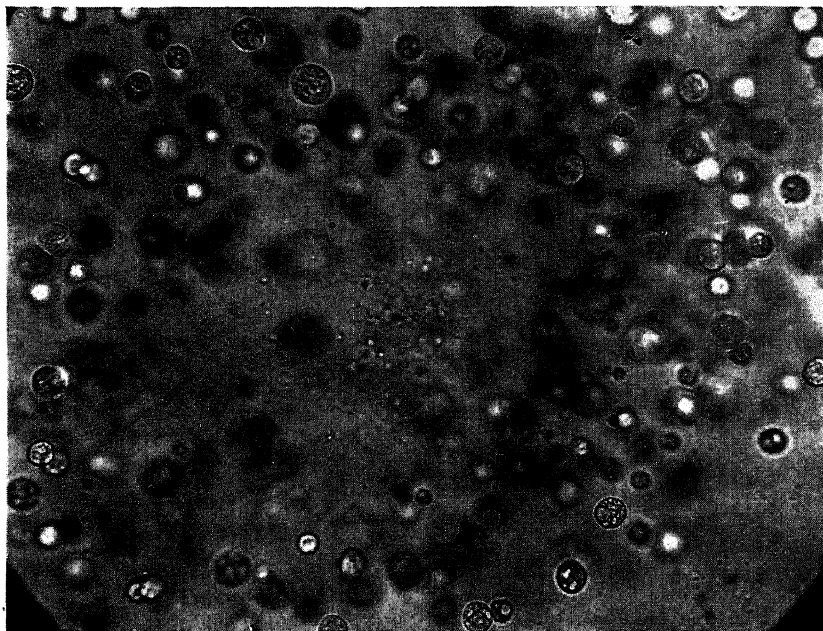


FIG. 4

FIG. 1. Microneedles stretching chromosome pair from egg nucleus of *Triturus pyrrhogaster*. Nuclear membrane has been removed. Chromosomes being dissected are 435 micra long. The stretched and looped chromosome below measures 810 micra. x 100.

FIG. 2. Diagram of chromosome pair in the half-size ovarian egg of amphibia. Paired granules are shown imbedded in plastic cylinder. Letters indicate various lateral loops, formed as outgrowths of the granules. Numbers indicate successive stages in formation of a nucleolus at a definite locus and its passage through the nuclear membrane.

FIG. 3. Stretching a chromosome. Note differential elasticity of various parts of the axis cylinder. x 240.

FIG. 4. Nucleoli at center of *Rana temporaria* germinal vesicle. Note relatively large mass of nucleolar material as compared to the central chromosomes. Pair of small nucleoli visible on chromosomes. x 860.

on a molecular level may take place. I wish to emphasize merely that these chromosomes may be stretched over 800 per cent or be shrunk to less than 20 per cent of their previous length without visible coils, and also to point out that Hauser found no oriented X-ray diffractive pattern of isoprene units in relaxed rubber fibers. It should also be recorded that most of Koltzoff's conclusions as to the structure of *Triton* chromosomes are not substantiated by observation of fresh material.

Our micro-dissection experiments consistently show that the

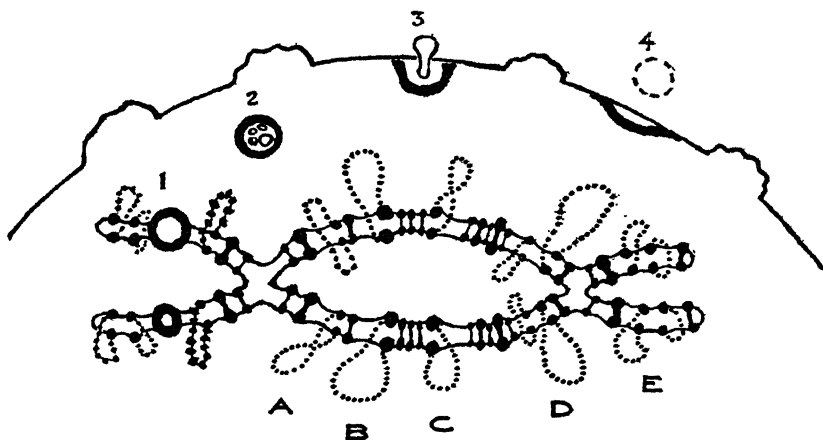


FIG. 2

chromosome is composed of a single longitudinal unit. This is in agreement with Chambers' pioneer observations on spermatocyte chromosomes of insects. However, the concept of a single chromonema is at variance with the classical tetrad theory. Such a difficulty is resolved by another fact which comes to light when strong X-radiation is applied—namely, that a latent cleavage plane runs throughout the length of the plastic cylinder. Under abnormal cellular conditions, application of 30,000 to the intact frog egg in Ringer solution results in occasional split or frayed chromosome fragments (Duryee, 1939). This indicates that the plastic cylinder, appearing as a single thread under the conditions of micro-dissection, actually possesses a molecularly frangible area which can split at this stage after drastic treatment like intense irradiation and after certain methods of fixation. It is well known that salivary

chromosomes possess similar latent fissures. These may be seen in certain hybrids carrying deletions and inversions, where they prevent complete somatic synapsis. Tetrad formation depends on factors at the molecular and therefore submicroscopical level.

Imbedded within the plastic cylinder are less elastic elements. These correspond best to the term "chromiole," although the definition for "chromomere" might also be fitted to them. No fundamental differences between those in frogs and salamanders have been found. Within a single nucleus some are more disc-shaped while others are irregularly rounded. Under oil immersion systems they appear within the cylinder material rather than merely affixed to its surface. Numbering hundreds per chromosome, they correspond approximately to denser segments of salivary chromosomes. It is to be emphasized that whatever irregularities of size, shape, and distribution appear in one member of a chromosome, duplicate differences show in the synaptic mate. No detailed map has yet been made, due to their extreme complexity, but such a study should be expected to show constant characteristics in all homologous chromosomes of a species. When chromosomes are stretched or swollen, in favorable locations chromiole granules can be observed as minute paired bodies lying within a viscous matrix. Each pair has a lateral loop attached. This paired condition strongly supports the concept of lateral chromosome reduplication. Examination of large numbers of chromosomes has led to the following hypothesis of loop formation, for which microphotographs illustrating each detail are available. Single granules divide into two fragments connected by a transverse bar. In the diagram (Fig. 2) short bars are indicated at the extreme right. Then intermittent growth from the fixed points within the chromosome results in a buckling and lateral displacement of each bar. While no specific chemical information as to the growth mechanism is available, lateral loops in all degrees of formation can be demonstrated. Presumably the process is analogous to inorganic crystal growth or possibly to virus reproduction. At all events the loops normally push out to an average distance of 5 micra from their originating granules. Usually a few in each urodele nucleus extend laterally about 16 micra and some even further. Measurements for frog lateral loops are slightly smaller. It is a mistake for cytologists to place



too great reliance on size measurement alone. We must bear in mind that most nuclear magnitudes can be changed by a factor of 2 with small variations in the cell medium, such as tonicity, colloiddally active ions and pH.

### MICRO-DISSECTION EXPERIMENTS

Experimental evidence is divided into two categories: microdissection and microchemical. The former were for the most part carried out in 0.1 M NaCl with 0.01 M KCl unbuffered. A primary experiment was to ascertain the relative elasticity of the plastic cylinder and to see whether the lateral loops were continuous with their internodal areas. *Triturus viridescens* chromosome segments seized at random gave the following percentage increases in length before breaking: 114, 340, 200, 210, 324, 905, 450, 275, 415, 610, 620, 312, 630, 525, 350, 110, 890, and 810 (Average: 450%). On the other hand, when the medium was acidified to pH 4.2, no segment could stand more than 85% stretching. A similar series of tests with *Triturus pyrrhogaster* chromosomes showed an average increment of 364% in the Ca-free Ringer, but only a 101% increase in 0.001 M  $\text{CaCl}_2$ . All segments tested averaged 12 micra between the points seized by the microneedles. In Ca-free Ringer a single chromosome was stretched over 100 per cent 125 times without breaking or altering its relaxed appearance. Stretching over 200 per cent tends to prevent the cylinder from shrinking all the way back to its original length. These experiments clearly show that amphibian chromosomes are highly elastic and fairly stable bodies.

In no case did the lateral loops open upon stretching. This is the critical experiment for the loop theory. They merely became relatively widely separated from one another, remaining as closed rings. Loops originating from adjacent chromomere pairs were separated up to 30 micra; on relaxation the plastic cylinder drew them together again. Such a stretched chromosome with unopened loops may have its longitudinal thread invisible. The fine thread always reappears on relaxation.

Various other dissection experiments on loops and granules were carried out. Individual loops were hooked over two microneedles and opened out. Small segments of single loops, chromosome cylinders and attached nucleoli were cut, compressed and stretched in different solutions. Buckling of chromosomes on

compression supports the cylinder concept. All experiments concurred in the principal result that side loops and longitudinal cylinder are differently constructed. Thus Painter's conclusion that abnormal branches or side loops represent reduplicated chromosomes must be considered erroneous.

If very dilute calcium ( $\text{CaCl}_2$  0.001 M) is present, isolated chromosomes exhibit somewhat different properties. As pointed out above they became more brittle, and they buckled more easily on compression. Both cylinder and loops tended to shorten, thicken, and become more refractive. In dilute calcium chromosomes lose stickiness, and slip readily over the shaft of a glass needle, to which they adhered when freshly opened from the nucleus. This fact confirms other preliminary tests that a nucleus is extremely low or lacking in calcium. It also supports my previous conclusions (1937) that inside the germinal vesicle chromosomes must be normally held apart by some sort of translucent gel system that prevents entangling.

An interesting property of chromosomes is that they are attached to one another at their cross-over points or chiasmata. This is apparent, upon seizing free ends of an allemorphic pair, and attempting to separate them with micro-needles. Another type of experiment is to insert needles into the lumen between chiasmata, and place a traction upon the points of juncture, as demonstrated in Fig. 1. Chromosome cylinders usually break before the chiasmata are separated. Such a relatively firm union is somewhat surprising, but may be observed to depend on the cementing and mucoid properties of the cylinder. No visible evidence of any quadruple exchange of chromatid fibers, such as called for by the *chiasmatype* theory, was ever gathered. If such an interchange of postulated fibers occurs at the chiasma, they must be smaller than can be observed with an oil immersion system. Direct observation indicates merely that cementing of plastic cylinder material is shared at the point of juncture.

As a corollary to these data it is indicated that in the living cell some local autolysis or digestion or other type of loosening must occur at the chiasmata to allow chromosomes to separate at meiosis. Such a deduction would be in line with the fact that genetically these loci are found to be less stable. A comprehension of the cementing properties of the plastic (or here viscous) cylinder should aid further understanding of chromo-

some behavior with regard to inversions, translocations, deletions, and synapsis. Nebel has brought forward diagrams showing how matrix material might aid in cementing broken ends together. Here these properties belong to the central chromosome itself.

A comparison of amphibian chromosomes with those in salivary glands of insects is not without significance. Especially since Calvin, Kodani, and Goldschmidt (1940) have recently shown that alkalinization treatments of the latter reveal paired connected chromomere granules with lateral branches imbedded in a mucoid cylinder. Geneticists like Hughes have shown in *Drosophila* hybrids that half-chromosomes merge with each other where possible, but that certain segmental areas with incompatible gene complexes remain apart, causing a fork in the chromosome cylinder. As previously pointed out, *Rana catesbiana* eggs heavily x-rayed (above 30,000 r) exhibit chromatids with partial longitudinal splits. With lower doses the evidence for splitting is less distinct. This was interpreted to mean that a latent cleavage plane traverses the length of the chromosome. However, it must be emphasized that longitudinal splitting is far less frequent than transverse fission, which I demonstrated in 1939 could be produced in egg chromosomes by heavy irradiation of only the whole cell. Except under these unusual environmental conditions of x-ray treatment, etc., the longitudinal filament always appears as a single elastic cylinder. It may be recalled that amphibian egg chromosome structure is closely parallel to the original description of Chambers for *Deisosteira* spermatocyte chromosomes, where the elastic cylinder was first dissected.

## CHEMICAL EFFECTS ON CHROMOSOMES

An extensive series of experiments on effects of ions of the Hofmeister series was carried out on loop structure as well as on other nuclear components. The accompanying table shows that their water solubility is enhanced by hydrative ions, but decreased by the opposite sort. In general loops are not dissolved by acids except in very strong concentrations greater than 0.7 molar. On the other hand they vanish quickly in low concentrations of hydroxyl ions wherever the pH is greater than 8.4. Lateral loops are readily attacked by properly adjusted

solutions of trypsin and pepsin. The cylinder is markedly more resistant to the latter. It is possible that normal nuclei contain chromosome disintegrants of the type of nucleases. It has frequently been observed that chromosomes from ovaries isolated several days, or from frogs long in captivity show deteriorative loops. Such conditions are simply variants, however, of the normal processes whereby the loops are removed from the chromosomes just prior to maturation.

RELATIVE COLLOIDAL EFFECTS OF ANIONS OF THE LYOTROPIC SERIES ON ISOLATED NUCLEI

(Sodium salts 0.1 M)

Anion	pH	Ground Substance Colloid	Chromosome Frame	Nucleoli	Chromosomes
Hydroxide 0.01 M	8.6	Swells, bursts	Expands Solvates	Dissolve immediately	Dissolve slowly
Succinate 0.3 M		Swells rapidly	Solvates	Dissolve	Hydrate, insoluble
Sulphide	8.4	Swells rapidly	Hydrates 3 min.	Dissolve progressively	Hydrate, Loops soluble
Iodide	7.2	Swells 10 min.	Hydrates, but not soluble	Swell, Evert	Hydrate, Loops soluble
Thiocyanate	7.4	Swells 20 min.	Hydrates, but not soluble	Swell, insoluble	Hydrate, Loops soluble
Taurocholate 1%		Swells 20 min.	Hydrates, but not soluble	Slowly fragment No. II insoluble	Hydrate, Loops soluble
Citrate	7.8	Swells slightly	Hydrates, but not soluble	Slowly fragment No. II insoluble	Hydrate, Loops soluble
Acetate	8.6	Swells slightly (few granules)	No change	No change	No change
Sulphate	5.7	Swells slightly	Hydrates, but not soluble	No change	Hydrate, Loops insoluble
Chloride	6.8	No change 15 min.	No change	No change	No change

An important and useful characteristic of the chromosome as a whole is its property of possessing a sharp iso-electric point. Following the technique of Churney and Klein, I have determined this to be for frog chromosomes  $\text{pH } 4.5 \pm 0.1$ . This point may be defined with respect to the chromosome as that pH at which it does not migrate in an electric field and at which it shows maximum contraction and maximum refractive index. The chromosome's I.E.P. is approximately 0.1 of a pH unit more alkaline than that of the surrounding nuclear colloid or chromosome frame. The measurement was estimated by watching the progressive darkening of the filament ahead of the converging precipitation area, I have described as the "I.E.P. ring." Any source of hydrogen ions in the proper concentration serves equally well, with an overlapping series of indicators, including phenol red, methyl orange, and alizarin red S and others.

Since isolated chromosomes in Ca-free Ringer are extremely hyaline and difficult to photograph, the illustrations in this paper were taken of unstained preparations adjusted to approximately pH 4.5. Of all methods for fixing, staining, or otherwise rendering chromosomes visible, this one is the least objectionable. Criteria for life and death do not apply to chromosomes. Mild realkalinization to pH 7.4—7.6 restores them to their original appearance. No experimental method is at hand to test whether they are still functional.

Turning now to the dynamic aspects of germinal vesicle chromosomes, we must recognize what all observers agree upon, namely, that lateral loops appear early in amphibian oogenesis and persist for considerable periods of time. In most species of frogs the loops reach maximum size in the second or third summer. Directly after ovulation the small eighth-size eggs remaining in the ovary already have large loops. These eggs presumptively give rise to the next season's batch. In Salamanders the time schedules are less certain, since eggs under optimum conditions may mature more or less continuously throughout the year. In any case it is clear that lateral loop development is achieved by a slow laborious process of chromosome synthesis. The normal fate of the loops, their number in the thousands, their origin from paired granules in the chromosome cylinder, all unite in indicating that gene units or groups of genes are manufacturing substances essential for the early embryo.

It has heretofore been considered that lateral branches of chromosomes were resorbed. Data controverting this hypothesis fall into three parts. First, the larger and older the eggs, the fewer and smaller are the remaining lateral loops. No one has offered better or more convincing evidence on this point than Rückert. Secondly, one can observe in the larger germinal vesicles a progressive increase in number of hyaline granules, approximately  $1\ \mu$  in diameter, in between and adjacent to the chromosomes. These are lateral loop fragments. Their increase in number is also noticeable in nuclei of eggs that have been isolated from the ovary for several days. It follows that nuclei must have some sort of autodigesting mechanism for removing the loops. Thirdly, we have the experimental approach. As shown in Table I, a variety of common anions, especially  $\text{OH}^-$ , rapidly strip off the loops, leaving the longitudinal thread. Facts such as these offer strong support to commonly accepted genetic theory, that master molecules or molecular templates, as genes have been called, remain imbedded in the axis cylinder ready to divide in meiosis and become distributed for work in some future cell.

### NUCLEOLAR PRODUCTION

Another type of chromosomal physiology that these studies have disclosed is the method of production of nucleoli. In amphibian eggs nucleoli are apparently produced successively during early and middle ovogenesis, because their number increases with age. However, nothing is known of the actual rates of these processes, since it is impossible continuously to observe chromosomes *in situ* for days or weeks. Table I shows how readily some ions of the Hofmeister series dissolve nucleoli. Many other reagents have been tested that agree in showing that these negatively charged bodies are susceptible to anion disintegration but resistant to cations. Hydroxyl ions and hydrogen ions in excess have sharply different effects, consequently nucleolar behavior can be used as a criterion for the penetration of these and other ions. It is important to note that merely changing the sodium chloride concentration can make nucleoli dissolve, fragment, or coalesce. It is a mistake to consider them as simply morphological units, since they behave and function as colloidal coacervates. Limitations of

space prevent a comprehensive discussion of nucleolar structure here, but no student of cytology should fail to realize that the exhaustive papers of Bungenberg de Jong and his co-workers on non-living colloidal coacervate systems have furnished the key to understanding the nucleolus. His model systems of complexes like gelatin plus arabinates are startling analogies to nucleoli and even may be mistaken under the microscope for them.

Nucleoli are produced at definite loci on three or four pairs of chromosomes. No significant differences between those of anurans and urodeles have been noted. Wherever a nucleolus is seen attached to a chromosome, another can be found at the homologous position in the synaptic mate. A growing nucleolus cannot be dislodged from the chromosome by a micro-needle without breaking the chromonema.

Nucleoli, ordinarily vacuolated, disintegrate in typical coacervate fashion. Hydroxyl ions, for example from .01 M  $\text{NaHCO}_3$  solution, begin by fragmenting the nucleolar membrane and thus allow the enclosed vacuoles to separate. Depending on subsequent coagulants or dispersing agents, the vacuoles swell and burst or coalesce into bizarre shapes. Structures familiar to cytologists under the term *amphinucleoli* can be formed in this way. Such reactions are so close to those described by Bungenberg de Jong that henceforth we should define an egg nucleolus as a coacervate formed by the interaction of a nucleolus producing locus in a chromosome with the surrounding colloid. This definition does not, of course, apply to many other types of nucleoli in other kinds of cells. Gersh has recently added important new data on the albumen content of nucleoli. He reports that their I.E.P. varies between younger and older eggs from pH 3.3 to 4.4, and confirms Brachet's finding no thymonucleic acid. I have shown that they stain supra-vitally with basic dyes and that phase reversals of the colored and uncolored portions can be brought about.

It remains only to point out that nucleolar volume produced by chromosomes during egg growth is considerable. Relative sizes are apparent in Fig. 4. Under high-power magnification, the central area of a full-size germinal vesicle is largely filled with nucleoli. The chromosomes in their non-contracted colloidal frame compose less than 1/1000th of the total nuclear

volume. Consequently an accumulation of nucleoli is evidence of chromosome function.

In the diagram (Fig. 2) is shown another detail—namely, that nucleoli can evert their contents through the germinal vesicle membrane. Whole eggs treated to alternating pH changes show clear nucleolar eversions. In each case the nucleolar membrane became incorporated with the nuclear membrane. It is presumable that the latter grows in this way.

Finally I would point out that we have here two concrete examples of how a nucleus functions in a cell. One is by producing lateral loops. The other is by nucleolar production. In both cases chromosomally elaborated substances are liberated normally into the egg cytoplasm at the time of germinal vesicle breakdown. Colloidal and chemical processes thus are cellularly related to descriptive morphology.

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## Radiation and the Cell Nucleus

*By*

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How radiation acts on living matter is of basic biologic importance. This is true, for with radiation the biologist has been able to exert some measure of control over the destiny of cells. Indeed, with this agent he has, during the past two decades, accomplished with notable success the experimental modification of cell behavior. Cells destined to follow a particular pattern of life have been caused to manifest a changed existence—even neoplastic and malignant growth.

As we look forward to still more complete control of cell function, we may profit by stopping to take note of how radiations such as X-rays and gamma rays act to induce biologic changes. Much attention has been devoted to the atomic physics of radiobiologic reactions and to the end results obtained in living test objects, but comparatively little has been given to the whole reaction and to the various kinds of reactions. It will be the purpose of this presentation, therefore, to set forth as complete a picture as possible consistent with the information now at hand, and at the same time to indicate the relationship these developments may have with our present impressions of cell activity. We shall first deal briefly with the nature of matter in general and how radiation acts to modify it, after which we shall consider at some length the question of how radiation affects living matter. Being aware that there are various elaborations of the detailed concepts of matter, we shall base our remarks on a somewhat simplified picture of the Bohr atom, about which there is little disagreement and which is fully adequate for the present discussion.

### I. MATTER IN GENERAL

All matter appears to be made up of atoms, most of which are held together in particular configurations to form mole-

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cules. Molecules, in turn, serve as building units to make up the morphological entities with which we come in contact in everyday life. Atoms are believed to be composed of a central nucleus which contains practically all of the mass, and which has a positive electric charge. Further, it is believed that negative units of electricity called electrons revolve in an orbital system about the nucleus.

The simplest atom is that of hydrogen (Figure 1 ( $H^1_1$ )). Its nucleus has a mass of one on the atomic scale and a single charge identical with that of the electron but positive in sign. This nucleus is called a proton. The normal hydrogen atom has a single orbital electron as indicated in the diagram. The net electric charge of the normal atom is zero; hence the positive charge of the nucleus must be just balanced by the negative charges of the electrons in the orbits.

Next in increasing atomic complexity after hydrogen is the atom of "heavy hydrogen," or deuterium. It has a mass of two but only a single positive charge on the nucleus and a single orbital electron (Figure 1 ( $D^2_1$ )). Its nucleus is called a deuteron. The deuteron apparently contains an additional particle of unit mass which, however, has no electric charge—a neutron.

The element next heavier than hydrogen is helium, Figure 1 ( $He^4_2$ ). The normal helium atom has a nucleus consisting of two protons and two neutrons. It must, therefore, have two orbital electrons. The nucleus of the helium atom is an alpha particle. All other atoms appear to be made up of combinations of protons, neutrons, and alpha particles with the proper combination of orbital electrons to insure electrical neutrality.

The total weight of the nucleus, which is the sum of the weights of the protons and neutrons, is the atomic weight (expressed in relation to oxygen, 16). The number of elementary charges on the nucleus, which is equal to the number of orbital electrons, is the atomic number and defines an element chemically.

All of the elements can be placed in a series or table (the Periodic Table) in which each element has an atomic number greater by unity than the one before it. As the nuclei become more complicated and more electrons are added, the development takes place in an orderly manner. In helium, the two electrons occupy similar orbits. In the next substance (lithium) a second group of orbits or shell appears, and successive ele-

ments add an electron in this shell until it holds eight, making, with the two in the innermost one, a total of ten (neon). With the next substance (sodium) atomic number 11, a third shell is added. This sort of development goes on to uranium (atomic number 92, atomic weight 238). One, and only one, element is known for every number in this series.

The chemical properties of an element are determined by the number of planetary electrons. Those in the outermost orbit are the valency group. They determine the chemical affinity of atoms (or chemical radicals) for each other. Chemical union apparently is brought about by the tendency of atoms to fill their outer orbits. This it would seem is accomplished by some kind of common sharing of the electrons in the outer orbits of the atoms. It is clear that if a firm chemical union is effected only when outer orbits are completely filled, only certain atoms or certain groups of atoms can be combined. To illustrate, take hydrogen ( $H_1^1$ ) and oxygen ( $O^{16}_8$ ). Hydrogen contains one proton and one planetary electron, as shown in Figures 1 and 2. Oxygen has eight neutrons, eight protons, and accordingly, must have eight planetary electrons (Figure 2). Obviously the addition of one hydrogen atom to oxygen does not furnish enough electrons to complete the oxygen's outer orbit, whereas the addition of two does complete it. Two hydrogen atoms may be united with oxygen in some such arrangement as shown also in Figure 2 to form the familiar water molecule. Figure 3 shows the possible arrangement of another familiar molecule ( $NaCl$ ). From these examples it is clear that in terms of protons, neutrons, and electrons, large organic molecules must be exceedingly complex. Further, although the information is incomplete as to the exact manner of the electron sharing, three points important to the present development are clear.

(1) Atoms tend to maintain an electrical neutrality between the nucleus and surrounding electrons; likewise, they tend to keep the innermost orbits filled to capacity, as far as the number of planetary electrons will permit.

(2) The chemical properties of an atom are determined by the number of planetary electrons, the valence depending on the number in the outermost orbit.

(3) Atoms (or radicles) are held together in groups to form molecules by the tendency to fill completely their

outer orbits with electrons by some kind of a common sharing of those in the valence groups.

## II. RADIATION

The term "radiation" has come to include two quite different concepts of energy transfer—one by means of waves, the other by means of particles.

Electromagnetic or wave radiation includes a wide range of types from radio waves to gamma rays. These are not associated with matter, but represent a propagation of energy by means of waves traveling in all cases at a speed comparable with that of visible light. Particle radiation, on the other hand, comprises various atomic and subatomic particles set into very rapid motion either by the action of electric fields or of radioactive sources. The energy content of these particles depends on their mass and speed. Electro-magnetic radiation may, in a sense, also be usefully pictured as consisting of particles of energy. In this case instead of a wave, the ray is called a photon, and the amount of energy associated with a photon is called its quantum. The energy content of a photon may be correlated with wave length as pictured by wave radiation; photons of high energy content correspond to relatively shorter wave length and high frequency whereas those of low energy content correspond to longer wave length and low frequency. Radiation, whether particulate or electromagnetic, acts on matter in three ways: (1) By causing thermal agitation; (2) by causing excitation; and (3) by causing ionization. These may now be discussed individually, and for purposes of illustration let us consider the action of electromagnetic radiation since its interaction with matter may also embody particle radiation reactions.

*Thermal agitation.* When sufficiently low energy photons (or particles) impinge upon atoms, they modify the direction of motion of these particles. Some energy is transferred which is taken up in the form of kinetic energy and manifested as heat. In view of the fact, however, that doses of radiation capable of producing far-reaching biologic change do not increase the temperature appreciably, it seems that this form of interaction is of lesser importance as far as radiobiologic changes are concerned.

*Excitation.* Photons (or particles) with somewhat higher energy content may cause an electron to be displaced from an inner to an outer orbit of the atom as illustrated in Figure 4. An atom having experienced such a change is said to be in a state of excitation or simply excited. Usually, however, atoms rarely remain long in the excited state, for electrons from the outer orbits drop into the vacancies created in inner orbits causing such atoms to emit energy as this happens. The transitory excited state of the atom is of interest in the present development, since the atom in this condition may have a greater opportunity for chemical change.

*Ionization.* Radiation with still higher energy content will cause electrons to be removed completely from atoms, thus producing a condition of ionization and a pair of ions, one member of which is the negatively charged electron (called secondary or recoil electron) and the other the positively charged atom (Figure 5). If the electron takes up the full energy of the incident radiation particle, the electron is said to be removed by photoelectric effect. If, however, the electron does not take up the full energy of the incident particle and a lower energy (longer wave length) photon emerges, the electron is said to be removed by Compton effect (Figure 5). In either case, the reaction may be pictured as the vacant inner orbit being filled by an electron from an outer orbit, the change therefore resulting in the loss of a valence electron. Should a free electron drop into the valency orbit from outside (recombination) no chemical union or change would be experienced (Figure 6). However, should other atoms with proper valence be attracted, chemical change would result and a new photo-product formed, an event of particular importance if it occurs in a vital part of a living system.

If different qualities or wave lengths of electromagnetic radiation are used, the physical reactions produced differ in certain important respects (Figure 7). The difference appears to be in the distribution of the ion pairs produced. The beginning of the trajectory of the secondary or recoil electrons has a distribution that varies with the amount of energy imparted to the electron. After the electron takes up a large amount of energy, it moves swiftly and the distribution of ion pairs is sparse (ion pairs per unit length of track is called specific ionization). As it loses energy and moves more slowly, the ion pairs produced

are relatively closer together. If the recoil electron receives a substantially smaller amount of energy at the beginning, it will move more slowly from the beginning and its trajectory will lack that part with ion pairs widely separated. Thus specific ionization appears to be essentially the same at the end of the recoil electron paths, but different at the beginning, depending upon the amount of energy imparted. Figure 7 makes clear a point not emphasized above, namely, that by far the greatest amount of ionization is produced by recoil electrons and not by the primary photons or particles.

To summarize, the changes produced in matter by radiation take place mainly in two steps: (1) ionization, presumably resulting in the loss of valence electrons, and (2) chemical change, this being mainly responsible for the important biological changes. Obviously biologic changes may result either from the loss of essential products or the influence of newly formed photo-products not previously present.

### III. LIVING MATTER

Living matter for the most part is made up of cells—living units having usually a distinct cell membrane, a semi-fluid cytoplasm containing a variety of formed bodies, and a nucleus containing a matrix and certain chromatin material (Figure 8). These units manifest the power of growth, adaptation, and reproduction—characteristics which in a general way distinguish them as animate objects.

Living protoplasm is a complex system consisting of water, electrolytes, protein, and other organic compounds—enzymes, carbohydrates, lipoids, hormones, growth agents, and the like. The present concept of the cell, therefore, is that it is an exceedingly complicated system of physico-chemical equilibria (acid-base, oxidation-reduction, electrolytic dissociation, permeability, etc.) which shift in orderly ways under the influence of controlling factors (chromosomes, environment, and perhaps other agents) to bring about the particular cell functions—secretion, contraction, conduction, support, proliferation, synthesis, differentiation, and the like, with which we are familiar.

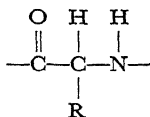
Two general divisions of cell activity are thus recognized: (1) functional, which includes all of the metabolic and nutritional activities of the cell, together with the special functions



(secretion, contraction, etc.) and (2) control, including the regulation and direction of all cell activity.

Significant in connection with the functional activity is the fact that there must be almost a constant supply of substrate materials available, together with an appreciable supply of catalysts, enzymes, and the like. This is in contrast to control activity where there are more fixed amounts of influencing factors such as hereditary substances (chromosomes and genes), and chemical substances such as growth agents, hormones, vitamins, and special mineral substances. Thus, in cases where there is an equal opportunity for molecules of either type of material to be injured or destroyed, the matter of availability, through concentration and replacement (by nutrition, synthesis, substitution, etc.), is likely to be of significance, depending on the biologic importance of the substances involved.

Proteins are of interest both as substrate materials in metabolism and as factors in cell control. Though of wide diversity they have one fundamental feature in common—they are composed of chains of amino acids joined in peptid linkage, each segment or link consisting of a nitrogen and two carbon atoms



These constitute the stem or backbone with a repeat design. One of the carbons has ketone oxygen linked with it, which is the remainder of a carboxyl group of an amino acid, whereas the other has attached to it as a sort of appendage, the amino acid residue and a side-chain R, which may be a simple hydrocarbon, an alcohol, or a base. Many of these segments are added together end to end to form the protein molecule. Since the side chains allow for a wide diversity of types, the possible variety of proteins is innumerable. Attention has been drawn recently to the similarity of the chain-like (or fibrous) proteins and cell chromosomes (Waddington (1), Demerec (2)). The thread-like appearance of the extended chromosome and the two-ended nature of its chromomeres, suggest that the chromosome consists of protein fibers arranged more or less parallel to its length. Geneticists, in fact, go so far as to say that chromosomes are composed of a fiber-like chromonema which is struc-

turally similar throughout the whole chromosome complex, and to which are attached various radicles; and that a segment of the chromonema with a number of radicles forms a molecular unit which is recognizable through its action as a gene. How well this most advanced concept of heredity may be fitted in with irradiation effects will be indicated later.

#### IV. THE ACTION OF RADIATION ON LIVING MATTER

Because of the discontinuous character of the action of radiation, small islands of chemical change are produced in the regions where energy quanta are absorbed. Further, because radiation such as hard X-rays and gamma rays penetrate uniformly to all parts of small living systems, these islands are produced at random in all the cell parts (membranes, cytoplasm, nuclei, chromosomes). There is at present no known mechanism by which any molecule or atom, whatever its setting, can be protected from an occasional alteration when subjected to radiations of the types in question. Hence in an organic system that is complex to start with, many different kinds of compounds are destroyed and likewise many new ones come into existence in a chaotic fashion. Obviously, any such hodgepodge of changes would make for disorganization in cell constituents associated with either functional or control activities. But, as indicated, the constituents in the two cases differ with respect to the amount that must be changed in order to cause significant differences.

Since substrate material is available for metabolic activity in appreciable if not excess amounts, it is to be presumed that functional activity would not be easily affected by radiation through destruction of substrate material. In any case, it appears that cells would not suffer permanent injury from such change. Likewise, it appears that many other elements associated with functional activity such as enzymes, catalysts, formed components such as membranes, mitochondria, cytoplasmic granules, etc., could suffer the loss of many isolated molecules without serious injury to the cell. Certain activity might be altered, but with continued replacement the cell would soon recover and again be normal. Actually, experimental findings, for the most part, bear out this generalization.

Scott (3) in a recent article brings together the literature dealing with changes in functional activity induced by radiation. Such processes as respiration, glycolysis, nitrogenous metabolism, enzymatic activity, etc., have been investigated. In all cases cited, except two,\* these processes were found to be little influenced even by large doses of radiation. We may thus eliminate from consideration this rather large class of reactions, since it seems to be of lesser importance so far as radiobiologic reactions are concerned. It is not to be implied from this, however, that important biologic changes cannot be produced in the functional activity of cells, but rather that for doses sufficiently large to cause changes in control activity of far-reaching biologic importance, functional activity is usually affected very little.

As mentioned, control activity in cells is attended by more fixed amounts of influencing factors. Hereditary substances are made up of particularly small amounts of highly organized materials quite specifically localized along the chromosomes. Hormones and growth agents are less localized and are not in such small amounts, but the influence they exert on biologic activity depends upon specific concentrations. Thus loss of small amounts of either genetic or growth agent materials may have profound biologic effects, and one begins to be aware of a reason why control activities of the cell are more readily modified by radiation than are the functional. Further insight into the nature of radiobiologic reactions perhaps can best be gained by considering the possible kinds of effects produced by radiation in homogeneous populations of cells.

## V. KINDS OF RADIOBIOLOGIC RESPONSES

When a homogeneous population of cells is irradiated with X-rays or gamma rays and the percentage of cells killed is plotted graphically as a function of the dose administered, curves such as those shown in Figure 9 may be obtained. Such curves have been derived through experimentation with a variety of kinds of cells and test organisms, and they range in shape from those which display a marked S-shape or skew character (Curve A) to those which are of the die-away type

\* Mottram (4) and Crabtree and Cramer (5). Scott, however, points out some reasons for believing that these findings may not constitute exceptions.

(Curve D). Obviously, such curves represent some kind of final reckoning of all the factors which act to bring about the final result, and an analysis of the different types of curves should lead to some interesting facts about the reactions involved.

At the outset, one is impressed by the simple character of the curves. They are not complicated by a series of inflections with varying ranges and amplitudes. This in itself suggests that the reactions must depend upon a fairly homogeneous change produced by the radiation, and that a close relationship must exist between the production of this change and the manifestation of the lethal effect.

With this in mind, we should like to postulate the various radiobiologic reactions that might be expected, taking into account various types of cell responses and presuming that a close relationship does exist between some particular initial effect produced and the final result obtained. At the same time we should like to present examples of responses which appear to be typical of the types postulated.

TYPE RESPONSE I. *Single Event Action*. The simplest conceivable kind of radiobiologic response is that in which the final result is produced by a single event, that is, one in which the change produced is always holistic in each cell and the result of the single event. Evidence that such reactions occur is suggested by the fact that what appears to be exponential dose-action curves like C and D, Figure 9, have been obtained in actual experiment. For example, if varying doses of X-rays are applied to uniform samples of bacteria and a count made of those that survive to form colonies on an agar plate, a curve showing the number killed as a function of the dose of radiation applied is obtained which resembles Curve D (Wyckoff (6), Lea et al (7), Lorenz and Henshaw (8)). A second, and perhaps more important example, is the production of mutations (mostly in *Drosophila*.) If radiation is applied to the sperm of adult males which are then allowed to inseminate normal females, a higher percentage of mutations will be found among the progeny of such individuals, and the quantitative relationship between dose of radiation administered and number of mutations produced gives rise to a curve resembling Curve C.\*

In these two cases, the curves are alike in that they appear

\* For recent summaries of this work see Patterson and Muller (9), Schultz (10), and Timofeeff-Ressovsky (11).

to have no threshold part, that is, no region at the beginning where little or no effect is manifest. It thus appears that no accumulation of events must occur before the effect begins to appear, a finding which indicates that the successful registration of a single event in a particular sensitive region is sufficient to cause cell death. The die-away character of the curves (more particularly in Curve D) indicates that for equal increments of radiation dosage, fewer and fewer organisms are killed as the reaction progresses. This is in accord with that which would be expected on the basis of purely random action since the chances of the successful encounter of an energy unit with a sensitive cell part drops off as the number of viable cells diminishes. Such a reaction, if not complicated by other processes, would proceed in accord with the Law of Mass Action, and the die-away curve would be strictly of the exponential type. The experimental curves obtained appear to be of this character. Let us therefore accept Single Event Action as a possible type for purposes of classification, leaving the questions of plausibility to be dealt with later.

TYPE RESPONSE II. *Multiple Event Action; Effect Manifest in Holistic Fashion.* In contrast to single event action is multiple event action—that in which an effect is manifest after an accumulation of quantum events in the living test object. By deduction it becomes apparent that the dose action curve will have different shapes and different meanings depending on whether the response is holistic in character or manifest by degree in each cell as the events accumulate. Here we shall deal with the former.

For this, experiments of our own on the production of multipolar cleavage in the eggs of the sea urchin, *Arbacia punctulata*, furnish an excellent example. If adequate doses of X-rays (around 20,000 r) are administered to sperm (or eggs or both) before fertilization, the first cleavage of the egg, when it appears, will be multipolar—that is, instead of the egg dividing into two equal blastomeres, as is normal, it divides into more than two. The curve showing how the number of organisms undergoing this type of change varies with the dose of radiation applied resembles Curve A, Figure 9. This curve is S-shaped, thus displaying a significant threshold, a measure of which, when quantitatively expressed, gives an indication of the amount of radiation which must be given before the

effect begins to be produced. The characteristic features of this reaction are that it is accumulative and that the effect, if it appears at all, is manifest completely.

TYPE RESPONSE III. *Multiple Event Action; Effect Manifest by Degree.* Interestingly, a graded type of response was observed in the same sea urchin material treated in the same way. If X-rays are applied to sperm which are then allowed to fertilize normal eggs and the eggs watched, it will be found that the interval between the moment of insemination and the time when 50 percent of the eggs have divided is definitely prolonged. Further, as the amount of exposure to X-rays is increased, the amount of delay is likewise increased. The curve obtained, showing how the amount of delay varies with exposure of the sperm to X-rays, has no threshold part and is of the die-away type. Thus, whereas it resembles Curve D, it obviously means something quite different here. In this case, the effect in each cell varies with the amount of radiation applied. The reaction is thus of the multiple event type with each event contributing to the amount of effect in each test unit. Whereas the exponential character of the curve in the former case suggested single event action, here it suggests that a single substance or a single homogeneous condition is being modified progressively in each cell.

These are the basic type responses visualized, as allowed by the assumption that the effect observed gives a direct measure of the initial irradiation effect produced and also that a uniform type of response is dealt with. The first type of action implies that the biologic change, whatever its nature, is caused by a single quantum event; the second, that a number of quantum events are required, but that the effect, when manifest, becomes so completely; and the third, that each quantum event contributes quantitatively to the amount of effect observed in each cell, whatever the nature of this effect may be.

Varied responses—those not uniform in character—may also be produced. Whereas one may select just one kind of change and study it to the exclusion of the rest and deal with it under the categories listed above, it sometimes aids in the analysis to deal with the variety of changes. In the present development such a consideration is especially suggestive. Two examples of the varied type of response will be given.

Radiation applied to frog's sperm before fertilization of normal eggs causes larvae to form which show a wide variety of

abnormalities. To mention only a few, some individuals show dorsal flexure of the tail, some lateral flexure, and still others ventral flexure; some show a marked edema while others show none; some show spina bifida and various degrees of twinning, while others do not. It is thus clear that the factors present in the sperm having to do with later development in the egg are affected in different ways, and further that any one of a variety of abnormalities produced may lead to death of the organisms.

The second example has to do with the killing action of X-rays on free living cells of yeast (*Torula cremoris*). If varying amounts of radiation are administered to identical samples of these cells, which are then put to culture on agar plates, it is found that the number of colonies which will appear diminishes as exposure to the radiation increases. In fact, what appears to be an exponential type killing curve is obtained. In accord with that which has been said, this leads to the presumption that single event killing action is in effect here and that when the lethal blow has been struck the cell fails to give rise to a colony. While this may be and probably is what happens, there is this interesting additional aspect. If one examines microscopically the cells which fail to give rise to colonies visible to the unaided eye, it is found that some cells do not divide at all after treatment, that others divide once, others twice, three, four, and some a good many times before succumbing to the irradiation injury. It is, therefore, apparent that death is not brought about the same way in each organism. Here, as in the case of the frog's sperm, it seems that the cells must contain a variety of sensitive regions, injury to any one of which may lead to death. This, in fact, seems quite plausible if we accept the advanced concepts of hereditary materials as described and also the mechanism of the induction of chemical change as set forth. Since hereditary elements or genes are located along the body of chromosomes like beads on a string, and since these may also be special side radicles of long chain-like molecules, it is easy to visualize how radiation quanta acting at random to split molecules may dislodge or dislocate one combination of factors in one case and quite a different combination in another. Thus, even though the same mechanism of action might be involved, quite different results would be obtained or the same result (such as death) obtained by different vital genes being destroyed or removed.

When varied types of responses, such as these, are encoun-

tered, they may be dealt with under the categories given above by doing one of two things: One, selecting a particular kind of effect and dealing with it to the exclusion of all the rest, or two, dealing with part or all of the changes as a homogeneous group. It will be recognized that it was the latter procedure which was used in connection with the yeast experiments and that doing so made possible the interesting correlation between the single event action suggested by the dose-action curve and the idea that death is produced by injury to any one of a variety of sensitive regions as indicated by the microscopical examinations.

It is thus apparent that responses have been obtained through experimentation which seem to typify each of the kinds of reaction postulated. While this is interesting, it can have no particular significance until the basic assumptions are examined for plausibility.

## VI. PLAUSIBILITY OF BASIC ASSUMPTIONS

It will be recalled that the classification as just outlined is based on the supposition that a close relationship exists between the irradiation effect observed and the initial quantum effect produced. It is necessary at this point therefore to inquire just how close this relationship is.

It has been emphasized repeatedly throughout this development that when living systems are exposed to radiation, no cell constituent can be excluded from the possibility of alteration. Our question squarely put, therefore, is whether in the vast array of changes produced in a cell there is a particular change or particular class of changes which leads directly to the end result under consideration.

Perhaps the strongest indirect evidence that there is such a close relationship is the fact that reactions have actually been observed resembling all of the different types that can be postulated. Referring to Type Response III, it appears that because of the unique character of the response, the curve can have no other meaning than that indicating some change induced directly by the radiation which varies progressively in each cell. By referring to Type Response II, it is also clear that irrespective of what interpretation is placed on the nature of the reaction, a threshold relationship exists between the effect produced and the dose administered. Thus, so far as these



features existing as realities are concerned, there can be little doubt, and since they form integral parts of the basic concepts of the postulation, the experimental findings may be taken as evidence supporting the postulation as a reality.

Whether cell changes, extensive as cell death, can result from a single quantum event as suggested by Type Response I, however, is quite another matter. Could it be proved beyond doubt that the dose-action curve in this case is actually exponential in character, it would be difficult, if not impossible, to offer any other interpretation. Fano, in a report now in press, points out the extreme difficulty one may have deciding whether the best curve which fits experimental data, with no more than the usual variation, is of the single or multiple event type. Yet on the outcome of such decisions rests a large part of the support for the idea of primary random action. In the light of Fano's findings, it seems proper to conclude, therefore, that whereas the quantitative data do not disprove that the curves are exponential, neither do they furnish really strong proof that they are of this type.

Fortunately, however, there is further information apart from that of the dose-action curves which lends support to the idea of a close relationship between initial effect and final result. First, there is the now abundant evidence that mutations (including the lethal variety) may result from chromosome aberration (deletion, inversion, translocation), and the fact that such aberrations are induced by radiation. In addition, there is the evidence that these changes are produced independently of the duration of exposure, of temperature during treatment, and of the metabolic activity during treatment. Mutations, by definition, are holistic in character and, as indicated above, the dose-action curve for their production appears to lack completely any threshold character. Now, should it be supposed that chromosome aberration is induced by secondary influence—say by some secondary chemical change in the cell, the temperature, growth rate, metabolic activity, etc., would be expected to modify the quantitative results obtained. Since, however, experimental findings indicate that they do not, such findings may be taken as evidence in support of primary random action.

Perhaps the strongest support yet available is that obtained by Sax (12), which combines indirect and direct evidence. Sax noted that if certain plant cells (*Tradescantia*) are irradiated during the resting stage when the chromatin material is in the

form of long single threads, the chromosomes upon subsequent examination are actually seen to be broken or fragmented, thus giving direct evidence of a discontinuous type of action. If, then, a plot is made of the percentage of cells containing single-chromosome breaks as a function of the dose of radiation applied, a non-threshold type curve is obtained. On the other hand, if a plot is made of the percentage of cells containing two breaks, a definite threshold type curve is obtained. Since the quantitative data supporting these findings were obtained under especially favorable conditions, and since the findings are strictly in accord with the predictions based on the idea of chromosome breaks being produced by single quantum action, they may be taken as strong evidence substantiating the idea of single event action.

To this point nothing has been said about the nature of the quantum event, that is, whether it consists of the production of a single ion pair, the passage of an electron, or the absorption of the total energy of a photon. The simplest conceivable kind of reaction is one produced as a result of a single ion pair being formed. From that which has been said above about molecular change being induced by the removal of a single electron and about the possible nature of chromosomes, there is reason to believe that biological change extensive as death may result from an event as simple as the production of a single ion pair. It was indicated that removal of an electron by ionization of a molecule may cause that molecule to split and undergo chemical change. Hence, in view of the fact that hereditary elements may consist of long chains of protein-like fibers, it may be presumed that an ionization at various points along the body of a chromosome may cause a break which would result in a deletion, or in the case of additional breaks, in inversion or translocation. Thus, whereas photon absorption and electron passage may act as unit events to produce biological changes, it seems quite possible that such changes may also result from single ion pair formation.

## VII. SUMMARY

In conclusion, therefore, a substantial body of evidence may be assembled in support of the concept that a close, if not

direct, relationship exists between certain radiobiologic results observed and the initial irradiation effect produced. As a consequence, the exponential dose-action curves, insofar as they are reliable, may be taken to indicate that biologic changes extensive as cell death may be induced by single event action—perhaps even by the production of single ion pairs.

Because of our work with sea urchin gametes, we have been able to deal with the quantum aspects when the effect is of the accumulative type in each cell. In considering the inferences which came as a result, our attention was attracted to the question of how many types of response could be postulated when the various kinds of end points are taken into account, and also whether responses have been observed which fit into each category. This led to the classification set forth above and to the bringing together of the various type responses presented.

Accepting then the idea of random quantum action, what does all this mean to biology?

First of all, it directs attention to the possibility of discontinuity reactions in cells—that is, to reactions in which isolated single events may produce biologic changes of far-reaching importance.

Secondly, it indicates the uniqueness of certain entities in cells, that these entities are of molecular dimension and that single entities of this type may in a large measure control the destiny of cells.

Thirdly, and most important, the irradiation technique provides a means of performing surgical operations on such entities with a degree of ultra microscopic fineness, and at the same time provides a means of studying their nature and behavior.

Finally, since this presentation is being made under the heading of "Radiation and the Cell Nucleus," we wish to mention that whereas it appears that the more important radiobiologic changes are produced in the cell nucleus, the most satisfactory considerations of radiobiologic reactions seem to come from dealing with the cell as a unit. This accounts for the somewhat broader aspects dealt with. Mention should be made also that the above considerations pertain only to free living cells, whereas the responses of cell groups or tissues comprise quite a different chapter which likewise must be taken into account when formulating rationals of radiotherapy or biologic studies.

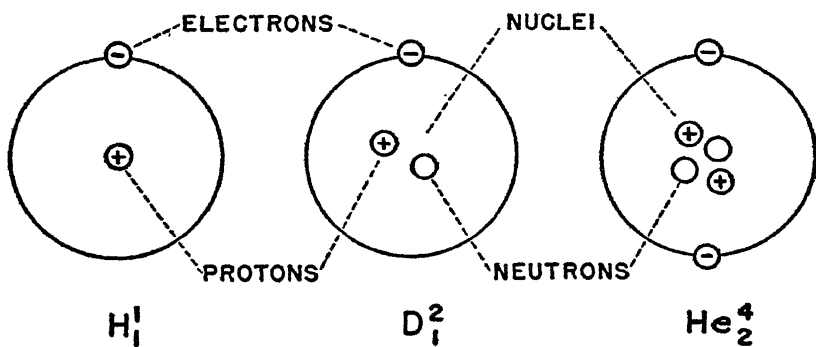


FIG. 1. Diagrams illustrating the hydrogen, deuterium, and helium atoms respectively.

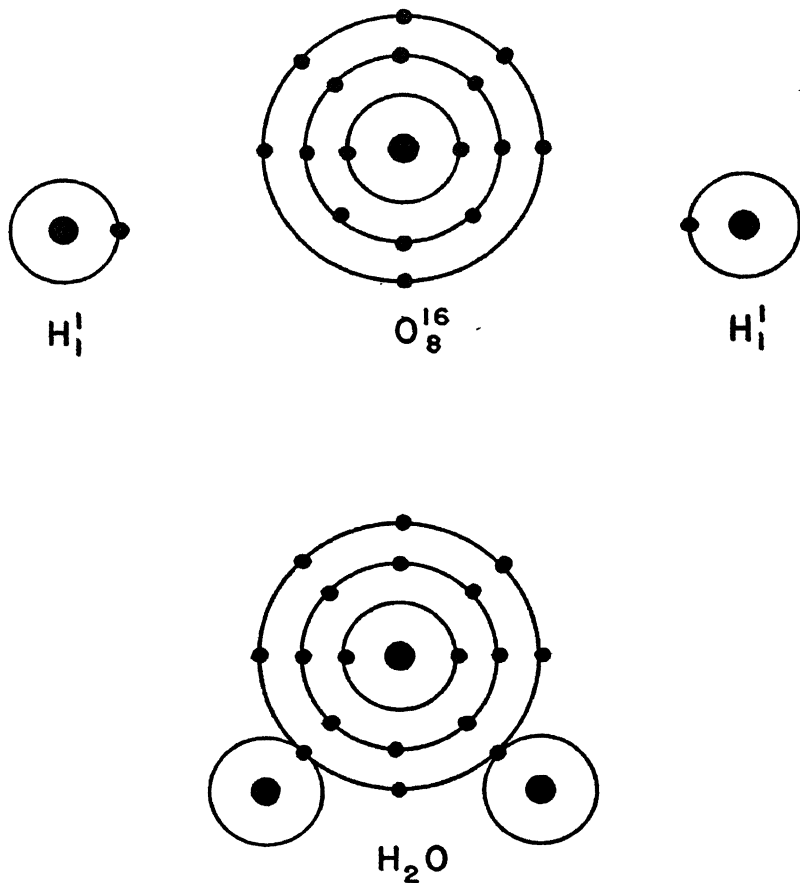


FIG. 2. Diagrams illustrating hydrogen and oxygen atoms and the water molecule.

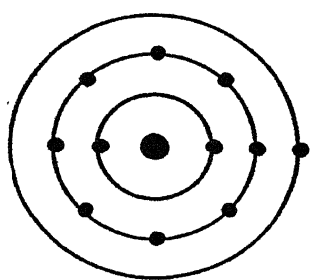
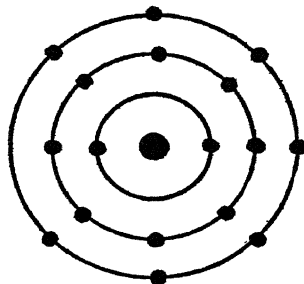
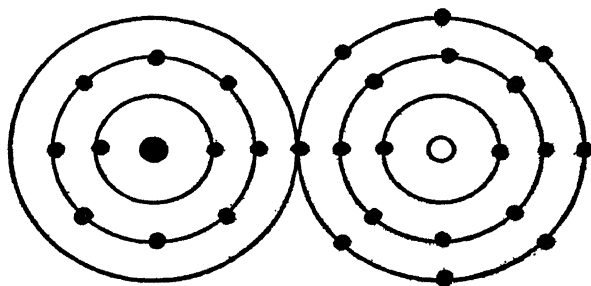

 $\text{Na}^{23}_{11}$ 

 $\text{Cl}^{35}_{17}$ 

 $\text{Na Cl}$ 

FIG. 3. Diagrams illustrating sodium and chlorine atoms and the sodium chloride molecule.

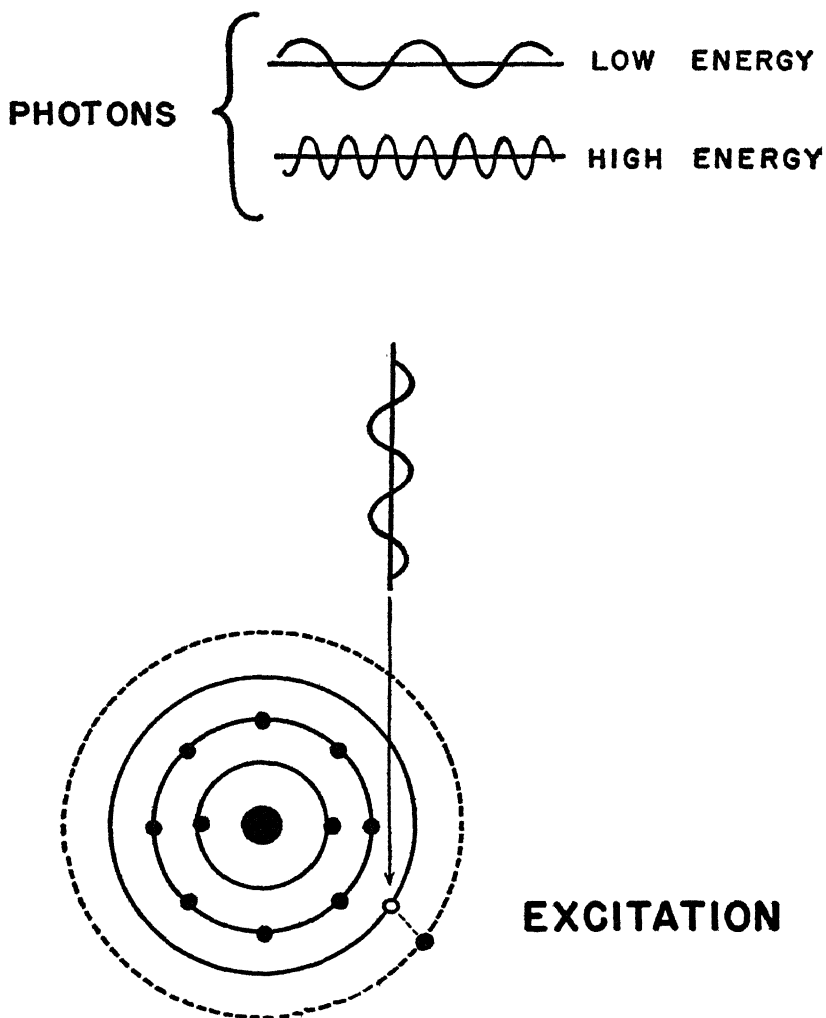


FIG. 4. Diagram illustrating the phenomenon of excitation in the sodium atom.

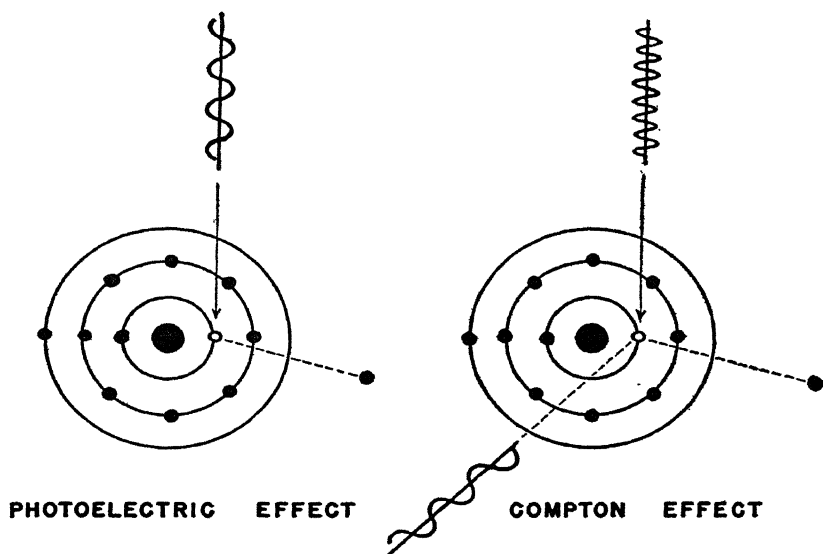


FIG. 5. Diagrams illustrating the phenomenon of ionization by photoelectric effect and by Compton effect in the sodium atom.

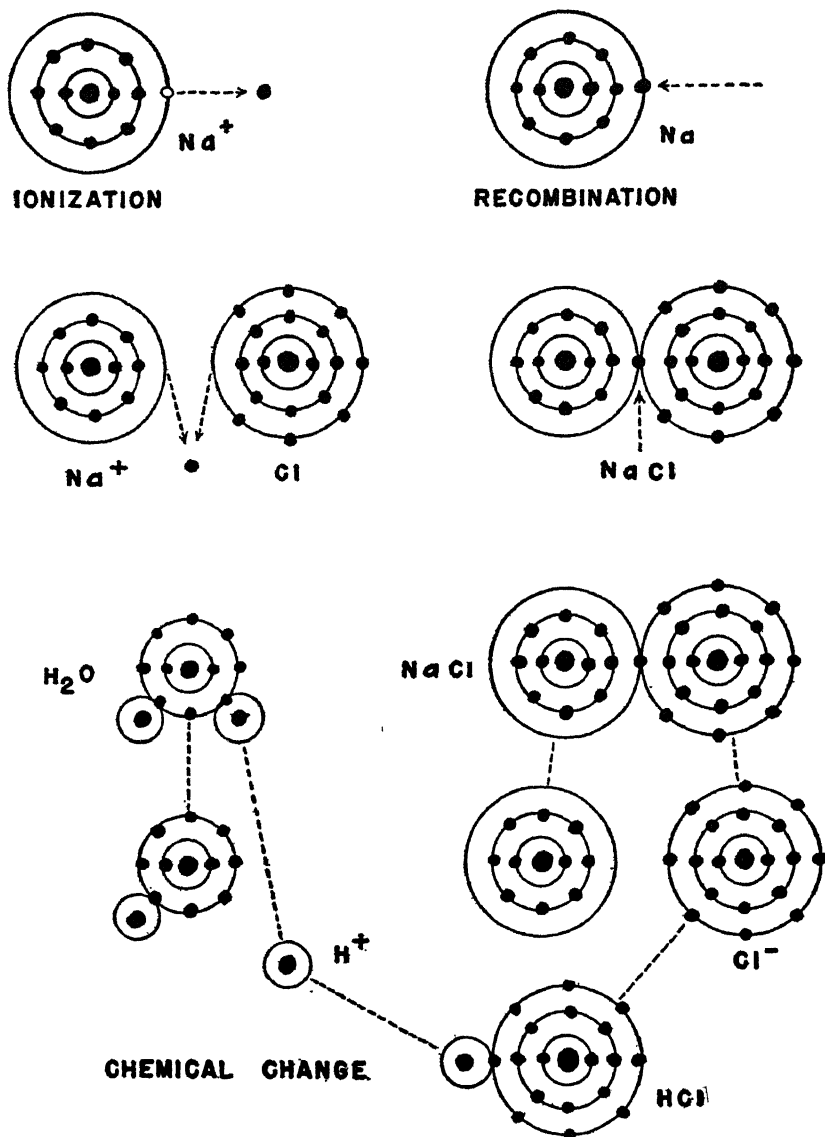


FIG. 6. Diagram illustrating (at the top) the phenomenon of ionization and recombination in the sodium atom, (in the middle) ionization, the consequent molecular splitting and the eventual recombination of the sodium chloride molecule, and (at the bottom) molecular splitting as may be produced by ionization of atoms in the water and sodium chloride molecules with a consequent chemical change to form a hydrochloric acid molecule.



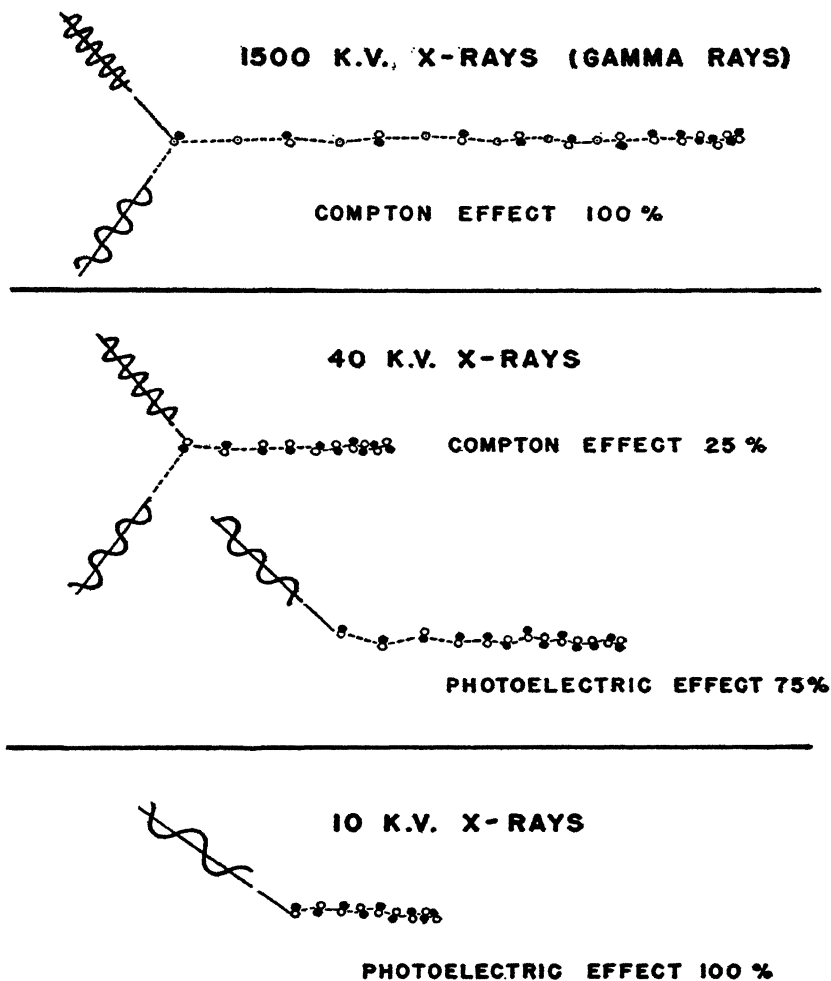


FIG. 7. Diagrams comparing the ionization produced along the trajectories of recoil and photo-electrons set in motion by photons having different energy contents.

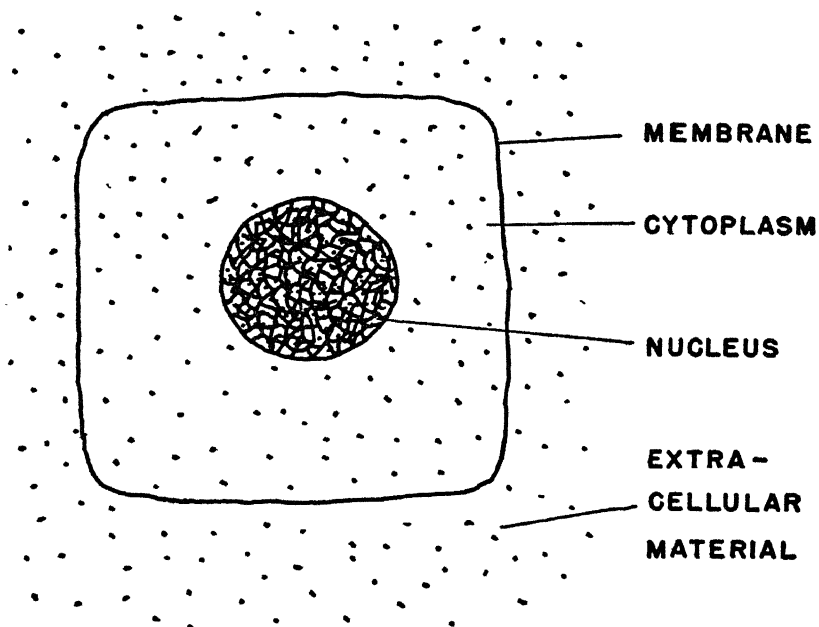


FIG. 8. Diagrams of a typical cell illustrating the fact that radiation acts indiscriminately on the various cell constituents.

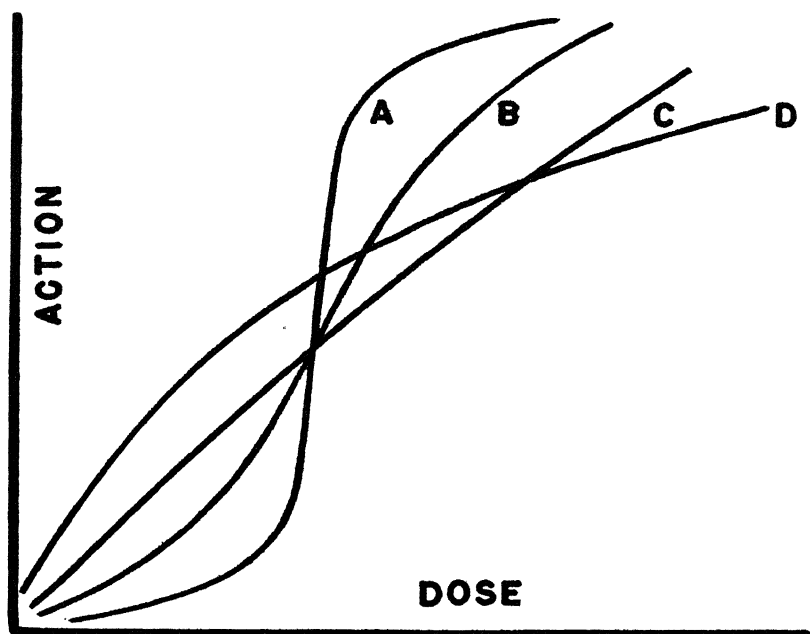


FIG. 9. Dose-action curves illustrating the progress of different types of radiobiologic responses.

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